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PROTEIN CONTENT OF REED CANARY GRASS ON PEAT SOILS[1]

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INTRODUCTION

Reed canary grass (*Phalaris arundinacea L.*), has been the subject of experiments for nearly 200 years, yet there is little agreement at present on either its nutritive value or its possible economic importance as a hay crop. From time to time during the past hundred years it has received more or less attention in this country without attaining importance as a crop, except recently and locally, and then mainly close to the north Pacific coast (*15, p. 230*[2]; *16, p. 16; 2, p. 3*).

Feldt, who has recently given the grass some critical study and is working on its improvement, speaks disparagingly of it as a deceiver because in the apparently great yields the content of dry matter and digestible protein is small (*7, p. 137*), and on sites adapted to it he recommends *Beckmannia erucaeformis* as a better meadow grass. Weber (*24*), on the other hand, in his recent (1928) monograph on *Phalaris arundinacea*, states that it provides an excellent hay, rich in protein, if cut at the proper stage of development, and under favorable conditions gives yields much in excess of those of the common grasses, even as much as 9 tons per acre of cured hay from three cuttings under the most favorable conditions in northeastern Germany.

The original purpose of the present study was to compare reed canary grass with timothy, considering both yield and protein content of the hay, as a meadow crop for peat soils that are very poorly drained or are subject to overflow, cutting both grasses at the usual stage of development, that is, early bloom, but the appearance of Weber's monograph directed attention to the importance of considering both soil conditions and time of mowing.

PREVIOUS PROTEIN DETERMINATIONS

Less than 30 previous determinations of the protein content of *Phalaris arundinacea* have been found. In Table 1 these are arranged in chronological order showing the percentages in the moisture-free grass. There seems no reason to suspect that any nitrogen fertilizers had been applied in the case of any of the first 20. Some of the samples analyzed were taken from meadows of almost pure stands of reed canary grass, but most of the first 13 consisted of few wild plants.

The earliest analysis is that by Gasparin in 1848. In what he designated "normal hay," but cut before flowering, he found 1.49

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[2] Reference is made by number (italic) to "Literature cited," p. 319.

per cent nitrogen—equivalent to 10.95 per cent protein on a dry basis (*10, p. 387*). The next analysis was by Ritthausen and Scheven, who found only 7.17 per cent protein. They had collected the plants on June 12, before bloom, but instead of analyzing at once kept them in water until after bloom (*13, p. 77*). About the same time Hosford in Massachusetts separated some of the plants into three parts—leaves, joints, and stalks without joints or leaves—and analyzed these separately. No mention is made of the stage of development of the plants (*9, p. 83*). His analyses showed: Leaves, formed 41 per cent of total dry weight with 1.13 per cent N; joints, 7 per cent of total dry weight with 0.45 per cent N; and stalks without joints or leaves, 52 per cent of total dry weight with 0.08 per cent N.

TABLE 1.—*Previously reported determinations of protein content of Phalaris arundinacea on a moisture-free basis*

Reference No.	Year	Analysts	Country or State	Protein content	Time of sampling or stage of maturity	Remarks
				Per cent		
1	1848	Gasparin (*10*)	France	10.95	Probably before bloom.	
2	1855	Ritthausen and Scheven (*13*).	Germany	6.17	After bloom	
3	1856	Hosford (*9, p. 82*)	Massachusetts	3.44		
4	1859	Arendt and Knop (*1*)	Germany	10.42	After bloom	Individual plants 80 to 90 inches high.
5	1876	Storer (*22*)	Massachusetts	11.06	June 13, before heading.	On part buds fully developed, 24 to 54 inches high.
6	1876			12.36	June 15, before heading.	Buds fully developed, 42 inches high.
7	1889	Hills (*12*)	Vermont	15.09		
8	1889	Cassidy and O'Brine (*4*).	Colorado	7.78		
9	1890	Wade and Patrick (*20*)	Iowa	16.88	June 10	Plants 36 inches high.
10	1890	Stebler (*21*)	Switzerland	6.90	July 26	
11	1893	Erwin (*6*)	Utah	12.64		
12	1893	Kellgren and Nilson (*14*).	Sweden	10.16	First part of July	
13	1894	Shepard and Williams (*19*).	South Dakota	8.20	June 20	
14	1908	Rindell (*17*)	Finland	6.93	In bloom	On sandy loam.
15				10.89	do	On reclaimed peat.
16				12.42	do	On light loam.
17				8.63	Ripe straw with seed removed.	Do.
18				12.40	10 days before bloom	On humus loam.
19				7.81	In bloom	Do.
20				9.73	do	On reclaimed peat.
21	1926	Feldt (*7*)	Germany	22.00	May 19, first cutting.	Fertilized on April 15 with 46 pounds per acre of N. supplied as Leunasalpeter.
22				11.33	June 9, first cutting	
23				7.35	June 26, first cutting.	
24				16.75	July 8, second cutting	After No. 21.
25				19.45	July 7, second cutting	After No. 22.
26				7.93	Sept. 20, second cutting.	After No. 23.
27				10.64	Sept. 20, third cutting	After Nos. 21 and 24.
28				10.24	Sept. 20, third cutting	After Nos. 22 and 25.
29	1928	Ermert (*5*)	do	10.48	Probably in bloom	

Soon after this Arendt and Knop collected plants from 80 to 90 inches high, that were past bloom, separated these into two parts, leaves, and culms with panicles, and determined the protein in each.

They found 16.50 per cent in the leaves and only 4.35 per cent in the culms with panicles (*1, p. 50*), but they do not report the proportion of each. If it be assumed that the leaves formed 50 per cent, a value suggested by the data in Table 9, the moisture-free hay would have contained 10.42 per cent protein.

Nearly 20 years later, in 1876, Storer in Massachusetts collected two samples of wild grass (Nos. 5 and 6 in Table 1) shortly before the heads appeared, with the individual plants varying from 24 to 54 inches in height, and found a slightly higher protein content than any previously reported (*22, p. 132*). The few later analyses in this country, all made 13 to 18 years after 1876, were of wild plants or of plants grown from the seed of these. The experiment stations of Colorado (*4, p. 88*), Iowa (*20, p. 457*), South Dakota (*19, p. 54*), Utah (*6, p. 255*), and Vermont (*12, p. 86*) each report one analysis. The percentage of protein found varied from 7.78 to 16.88 per cent (Nos. 7, 8, 9, 11, and 13 in Table 1).

Stebler (*21, p. 87*) of Switzerland found 6.9 per cent in the hay.

In the report on an investigation of the forage plants of Finland, in 1908, Rindell included analyses of seven samples of *Phalaris arundinacea* (Nos. 14 to 20 in Table 1). From the analysis of two samples from the same meadow, Nos. 18 and 19, he concluded that if the grass had been cut somewhat before bloom it would have been richer in protein. His analyses gave the highest protein values reported from Europe previous to the recent work of Feldt, but these were exceeded by those already reported from Vermont, 15.09 per cent, and from Iowa, 16.88 per cent.

During the following 20 years the grass appears to have received little attention, but in February, 1926, Feldt, of Königsberg, speaking before the Zentral Moor Kommission of Germany, pointed out that new determinations of its protein content should be made and suggested that through breeding and mowing several times in a season the grass could be made a more valuable forage plant than it is at present (*7, p. 137*). At a meeting of the same organization in December of that year he reported the analysis of eight samples, Nos. 21 to 28 in Table 1 (*8, p. 98*). The grass was grown on a loamy sand, rich in organic matter, and had been fertilized on April 15, 1926, with 200 kilograms per hectare of Læunasalpeter (equivalent to 290 pounds per acre of sodium nitrate). Three plots or tracts were mowed on different dates: No. 1 on May 19, July 8, and September 20; No. 2 on June 9, July 7, and September 20; and No. 3 on June 26 and September 20, there being no third growth to mow.

The protein content of the first cutting decreased as the age of the grass increased, namely, from 22 per cent on May 19 to 7.35 per cent on June 26. The second cutting, when made at the end of 28 days, showed 19.45 per cent; when made at the end of 50 days, 16.75 per cent; but when delayed for 98 days after the latest first cutting, it fell to 7.93 per cent. The two samples from the third growth, cut 75 days after the second, contained only 10 per cent protein. It should be stated that while a nitrogen fertilizer had been applied to all the plots in the spring there had been no later application. The samples analyzed were not of wild plants, nor from the commercial seed of Germany, but from a leafy strain, Massenauslese, developed by the Mooramt, and Feldt warns against assuming that the above protein

percentages from this strain, rich in leaves and poor in culms, would be found to apply to the wild strains of *Phalaris arundinacea*, which are almost always rich in culms. He expressed the opinion that most of the reed canary grass seed now on the market should not be sown on meadows from which a protein-rich hay is desired. Weber, in his monograph of which the preface is dated July, 1927, states that he had hunted in vain for analyses made of the hay harvested at the proper time, the one coming nearest being that by Rindell (*17*) mentioned above (No. 18 in Table 1). Evidently he was not acquainted with the paper by Feldt (*8*) which had been presented before the Zentral Moor Kommission six months before but was not published until much later.

Ermert (*5, p. 175*), of Königsberg, in a study of the forage value of hays in 1928, reported the protein in the hay from seven grasses all cut at the same stage of maturity, but what that was he does not state. The reed canary grass contained 10.48 per cent protein in the dry matter and the timothy 9.82 per cent.

EXPERIMENTAL DATA

SOURCE OF SEED

The samples of grass analyzed in this study were from plants grown from seed secured from three different sources. The main supply was of the Randowbruch strain of *Phalaris arundinacea*, purchased in the spring of 1927 from the meadow and pasture section of the Verein zur Förderung der Moorkultur im Deutschen Reiche, and referred to below as the German seed. About the same time, through the kindness of A. Kirssanoff, of Leningrad, small quantities of seed were secured from four Russian agricultural experiment stations, namely, those at Marussino, Moscow, Detskoe Selo, and Beresotochosk, the largest amount from the first. Lastly, there was seed from the Lake Madison district, 60 miles southwest of St. Paul, Minn., where a considerable acreage of the grass has been slowly developed from seed purchased by a local farmer from a Wisconsin seed company 30 years ago.

In presenting the results the data are grouped according to the source of the seed, although it was no purpose of the study to attempt the selection of a superior strain. The samples analyzed consisted of the entire aerial portion of the plants, cut off about 3 inches above the surface of the ground, taken to the laboratory, dried at about 50° C., finely ground, and finally dried at 100° C. before analysis. The percentages of crude protein reported in the tables are on the moisture-free basis and not on the hay, which is ordinarily assumed to carry 15 per cent moisture.

GRASS FROM MINNESOTA SEED

On September 27, 1926, a sample of the second growth was collected near Lake Madison from a meadow in which the first growth had been cut early in July, just after the seed had ripened. This aftermath was about 27 inches in height and contained 14.5 per cent protein. None of the plants in this, or any of the second and third cutting samples reported in this paper, showed any sign of forming heads.

AT COON CREEK EXPERIMENTAL FIELDS

Some of the seed that had been gathered from the above field and a small sod were taken to the Coon Creek peat experimental fields, 20 miles northwest of St. Paul, where on October 6, 1926, the sod, divided into four parts, was set out in rows. On the same day part of the seed was planted in five 5-foot rows 36 inches apart, the rest being sown a year later. Plants appeared early in the spring of 1927, and during the summer made a vigorous growth. On two of the rows they were harvested twice that season. On the first date, August 3, they were about 27 inches high, had not yet formed heads, and contained 17.31 per cent protein, while the second growth, cut on November 4, was about 12 inches high and contained 13.94 per cent protein. The plants in the three remaining rows were cut only once, on November 4, by which time they had reached a height of about 33 inches, and put forth a few heads, and carried 11.06 per cent protein. (Table 2.)

TABLE 2.—*Protein content of reed canary grass from Minnesota seed, grown in rows 36 inches apart on Coon Creek peat experimental fields, 1927–28*

Reference No.	Date of sampling	Protein content on a dry basis	Cutting	Average height of plants	Stage of maturity	Remarks
	1927	*Per cent*		*Inches*		
2	Aug. 3	17.31	First	27	No sign of heads	Seed sown in October, 1926.
3	Nov. 4	13.94	Second	12	do	Aftermath of No. 2.
4	do	11.06	First	33	A few heads formed	Sown at same time as No. 2.
	1928					
5	June 7	15.88	do	38	A third of heads out	From same rows as No. 4, mowed only once in 1927; seed sown in October, 1926.
6	July 23	21.94	Second	18	No sign of heads	
7	June 14	15.75	First	45	Most of heads out	
8	Aug. 17	14.25	Second	25	No sign of heads	
9	June 27	10.69	First	54	All in head	
10	Aug. 17	13.00	Second	25	No sign of heads	
11	June 7	13.81	First		No heads out	From same rows as Nos. 2 and 3, mowed twice in 1927; seed sown in October, 1926.
12	July 23	20.81	Second	15	No sign of heads	
13	June 14	14.82	First	36	A few heads out	
14	June 27	10.56	do	51	Beginning to bloom	
15	Aug. 17	14.06	Second	16	No sign of heads	
17	July 21	20.00	First	23	No sign of heads, south plot.	Seed sown in October, 1927.
18	Aug. 17	15.66	Second	29	do	
19	July 21	19.92	First	23	No sign of heads, north plot.	
20	Aug. 17	21.19	Second	27	do	
21	do	17.19	do	23	No sign of heads	From sods transplanted in October, 1926; first growth, 50 inches high, cut July 6 after seed had ripened.

In 1928 those of the rows from seed sown in October, 1926, that had been mowed only once in 1927, were divided into three lots and the first cutting made on June 7, 14, and 27, respectively. On the first date about 30 per cent of the heads had appeared, on the second about 60, while on the last the grass was fully in head and beginning to bloom. The first sample contained 15.88 per cent protein, the second practically the same—15.75 per cent—but the third only 10.69. (Table 2.)

The rows from seed sown at the same time as the preceding, but mowed twice in 1927, were similarly divided into three lots and harvested on the same three successive dates. The grass from the

first contained 2 per cent and that from the second 1 per cent less protein than the corresponding samples from the rows cut once in 1927, but on the latest date there was practically no difference.

The second cutting from the parts first mowed on June 7 was made at the end of 46 days, and contained 21.94 and 20.81 per cent protein, while that from the other lots, all sampled on August 17, contained only from 13.00 to 14.25 per cent.

On October 19, 1927, the remainder of the seed secured from the Lake Madison district in 1926 was sown at Coon Creek in rows 3 feet apart on two small plots adjacent to the first. No plants appeared above the surface until the following spring. On July 21, when the plants were about 23 inches high, but showed no sign of heading, both plots were mowed. The percentages of protein in the two samples were 20.00 and 19.92. A second cutting was made on August 17, by which time the aftermath was taller than the plants had been when mowed four weeks before. The protein content on one plot was 15.66 and on the other 21.19 per cent, with an average of 18.42. The difference of 5.5 per cent between the two, which had received the same treatment, would suggest that there was a more liberal supply of available nitrogen in the soil of one of the plots.

The transplants were not sampled in 1927; in 1928 the first growth was allowed to ripen seed and the straw was cut on July 6, but no analysis of it was made. The aftermath, cut on August 17, was 23 inches high and contained 17.19 per cent protein. (No. 21 in Table 2.)

On the whole, the protein content of the 20 samples of grass reported in Table 2 was high, averaging 15.88 per cent for all, with a maximum of 21.94 and a minimum of 10.56. The average for all samples of the first cutting was 14.98 and of the second, 16.89 per cent. No nitrogen fertilizer had at any time been applied, but the soil is naturally so rich in available nitrogen that cultivated and grain crops on it respond to nitrogen fertilizers only when the soil is cold, as early in the spring. Furthermore, the plants had the advantage of being in short rows 3 feet apart and surrounded by bare fallowed soil. The spaces between the rows also had been kept free from all vegetation except the underground stems of the reed canary grass. The general effect of these conditions was to assure the grass an unusually liberal supply of nitrogen.

In the Lake Madison District

As previously mentioned, there is a considerable acreage of reed canary grass meadow on farms in the vicinity of Lake Madison, Minn. It is also found growing along the public roads, being especially abundant where roads cross tracts of peat or muck. The meadows, on all of which it had been sown broadcast, in most cases from three to six years before, have a peat or muck soil, well provided with lime and with a supply of available nitrogen at least as abundant as that of the peat at Coon Creek. In the season of 1928, 26 samples of first, second, and third growth from this district were analyzed. (Table 3.) The first 10 samples were collected on June 17, when in most places the grass was more or less headed out but not yet in bloom; the next 7 on June 29, when nearly all the meadows were in bloom; the remaining 9 represent the second or third cutting.

TABLE 3.—*Protein content of reed canary grass grown in the Lake Madison district in 1928*

[The meadows had been seeded three years or more]

Reference No.	Date of sampling	Protein content on a dry basis	Cutting	Average height of plants	Stage of maturity	Remarks
		Per cent		*Inches*		
22	June 17	13.19	First	19	A few heads out	Farm A, field 1, very thick stand.
23	do	10.69	do	24	do	Do.
24	do	14.22	do	36	All in head	Farm A, field 1, adjacent to corn.
25	do	14.75	do	29	A few heads out	Farm A, field 2, younger meadow.
26	do	12.06	do	28	All in head	On highway beside farm A.
27	do	12.00	do	38	do	Farm B, meadow.
28	do	11.75	do	36	do	Farm C, meadow.
29	do	13.00	do	24	do	Isolated clump on roadside.
30	do	15.25	do	34	do	Do.
31	do	10.00	do	48	do	In roadside ditch.
32	June 29	8.56	do	46	In bloom	Farm D, meadow.
33	do	9.13	do	46	do	Farm E, meadow.
34	do	11.88	do	48	do	Farm B, meadow.
35	do	14.50	do	48	do	Farm F, meadow.
36	do	8.00	do	48	do	Farm F, another meadow.
37	do	10.31	do	48	do	Farm G, meadow.
38	do	15.75	do	30	Partly in head	Farm H, meadow.
39	July 25	19.75	Second	13	No sign of heads	Farm B, unfertilized.
40	do	19.63	do	13	do	Farm B, P supplied on June 29.
41	do	18.56	do	13	do	Farm B, P+K supplied on June 29.
42	do	18.44	do	13	do	Farm B, N+P+K supplied on June 29.
43	Aug. 20	13.00	do		do	Farm B, unfertilized.
44	Sept. 25	15.06	do		do	Farm I, first growth, cut for seed.
45	Oct. 1	25.25	Third	11	do	Farm B, second growth, mowed August 20.
46	do	22.13	do	12	do	From roadside near farm B.
47	(1)	14.56	Second		do	From farm I.

[1] About October 1.

On the oldest field sampled, a small shallow bog that had been seeded in 1919, samples were taken from three places, the first where the stand was very thick and the growth comparatively short, about 19 inches, with only an occasional head appearing, the second from an equally thick growth with plants about 5 inches taller and with an even smaller number in head. (Fig. 1, A and B.)

The third sample was of plants from the extreme edge of the meadow, forming the border of a field of corn that had been kept free of weeds and so was equivalent to a fallow. These plants were much darker in color, a foot taller than the preceding, and all in head, although not yet in bloom. (Fig. 1, C.) Notwithstanding their being more mature they carried from 1 to 3.5 per cent more protein than the preceding two samples. No. 25 (fig. 1, D) was taken as representative of another and larger field on the same farm, also on peat soil, but seeded five or six years after the first. The plants, about a tenth in head, were taller than those of No. 23 (fig. 1, B), but the protein content was higher than in any of the preceding three; that is, 14.75 per cent. A few hundred yards from the first meadow, on mineral soil forming the shoulder of a highway, an isolated clump of the grass was found with bare soil extending from 3 to 5 feet on all sides. On this (fig. 1, E) the heads were fully out but not in bloom, and the protein content was 12.06 per cent.

Nos. 27 and 28, with the plants fully in head, were taken from meadows on two farms a few miles distant. They had a protein content of 12 and 11.7 per cent, similar to that of plants in the thick interior of the first meadow. On a peaty roadside near the last field there were two isolated clumps of the grass, both fully in head and very different in appearance, although only 5 feet apart. One contained 13 per cent and the other 15.25 per cent of protein. The tallest plants seen in the district were found growing near those on peat soil in a roadside ditch. They were 4 feet high, fully headed out, but not in bloom, and contained only 10 per cent of protein (No. 31 in Table 3).

FIGURE 1.—Samples of first-growth reed canary grass collected on June 17 from a farm in the Lake Madison district, Minn. A (No. 22), From thick stand in interior of an old meadow; average height, 19 inches; protein content, 13.19 per cent. B (No. 23), From thick stand in interior of an old meadow; average height, 24 inches; protein content, 10.69 per cent. C (No. 24), From edge of same meadow bordering on clean, cultivated land; average height, 36 inches; protein content, 14.52 per cent. D (No. 25), From a younger meadow; average height, 29 inches; protein content, 14.75 per cent. E (No. 26), From shoulder of the adjacent highway; average height, 28 inches; protein content, 12.06 per cent. Numbers correspond with those in Table 3

The samples collected on June 29 were from seven farms, each sample taken as representative of the crop on the whole meadow. On part of the fields haying was in progress at the time, and on all except the last, No. 38, the plants were in full bloom. The protein content ranged from 8 to 15.75 per cent. No. 38 had a protein content of 15.75 per cent, but in this the plants were only partly in head, the field probably having been pastured early in the season.

On June 29 various fertilizers were added to plots laid out on a meadow from which the hay had just been removed and from which a sample (No. 34) was taken. These plots were sampled on July 25, when the second growth was 25 inches high. The protein content varied from 18.44 to 19.63 per cent.

A sample of the third growth on this field, taken on October 1, when the grass was about 12 inches high, contained 25.25 per cent protein, while a sample of about the same height taken from a near-by roadside contained 22.13 per cent (Nos. 45 and 46).

The average protein content of the 10 samples taken on June 17, while the grass was only partly in head, was 12.69 per cent, that of the 7 taken 12 days later, when the grass was in bloom, was 11.16 per cent, while the 9 samples of aftermath averaged 18.49 per cent.

This would give an average of 14.29 per cent for all 26 samples from the Lake Madison district. The average protein content of the crop for the entire season would be much below 14 per cent because of the comparatively small proportion that the aftermath formed.

GRASS FROM RUSSIAN SEED

The four lots of Russian seed mentioned above were planted at Coon Creek on June 15, 1927, in rows 36 inches apart. On November 4 the Marussino grass was sampled and found to contain 12.75 per cent protein. In 1928 a part of the rows from this source and all of those from the other Russian sources were allowed to ripen seed, not being mowed until July 6. The plants from all four lots appeared very much alike. The protein content of the first growth, freed of ripe seed before analysis, was low, ranging only from 6.94 to 8.31 per cent. (Table 4.) The aftermath, sampled on August 17, contained from 14.25 to 18.63 per cent. The relative richness in protein of the second cutting bore no distinct relation to that of the first. Thus the Beresotochsk ranked highest in the first cutting and lowest in the second, whereas the Marussino, which was next to the lowest in the first cutting, was highest in the second.

TABLE 4.—*Protein content of reed canary grass from Russian seed, sown in rows 36 inches apart, on Coon Creek peat experimental fields on June 15, 1927*

Reference No.	Date of sampling	Protein content on a dry basis	Cutting	Average height of plant	Stage of maturity	Source of seed
	1927	*Per cent*		*Inches*		
48	Nov. 4	12.75	First		No sign of heads	
	1928					
49	July 6	7.19	do	58	Ripe straw, without seed.	Marussino Experiment Station, Tambov Government.
50	Aug. 17	18.63	Second	22	No sign of heads	Do.
51	July 6	8.31	First	58	Ripe straw	Beresotochsk Experiment Station, Poltava Government.
52	Aug. 17	14.25	Second	20	No sign of heads	Do.
53	July 6	6.94	First	58	Ripe straw	Plant breeding station, Detskoe Selo, Leningrad Government.
54	Aug. 17	15.88	Second	16	No sign of heads	Do.
55	July 6	7.44	First	58	Ripe straw	State institute of meadow management, Moscow Government.
56	Aug. 17	17.44	Second	20	No sign of heads	Do.

Three rows of Marussino plants were mowed on June 18, no sample being saved for analysis, and divided into 11 parts for second and third cuttings. Beginning July 13, these were sampled and mowed the second time at 3-day or 4-day intervals, the latest mowing being on August 24. The third cutting on each was made from 49 to 53 days after the second, the earliest being on September 4 and the latest on October 9. Accordingly, samples of the aftermath were collected about twice a week during the last three months of the growing season. (Table 5.) The protein content of the 11 samples of second growth of Marussino averaged 18.86 per cent and ranged from 16.31 to 22.56 per cent without any distinct dependence upon the time of the cutting, the minimum being found at the end of 46 days and the maximum 10 days later. The growth of the plants was not vigorous. At the end of 26 days they were about 16 inches high,

and six weeks later they were only 6 inches taller. With the third growth the average protein content, 15.56 per cent, was about 3 per cent lower than with the second and the variations were narrower.

TABLE 5.—*Protein content of reed canary grass collected in 1928 from Marussino and Randowbruch seed, sown in rows 36 inches apart, on Coon Creek peat experimental fields on June 15, 1927*

[All of Nos. 71 to 114 from rows mowed first time on June 18]

Reference No. of—		Date of sampling	Protein in hay from—		Cutting	Average height of plants from—		Stage of maturity	Growth period
Russian seed	German seed		Russian seed	German seed		Russian seed	German seed		
			Per cent	*Per cent*		*Inches*	*Inches*		*Days*
57	58	May 28	17.75	19.31	First			No sign of heads	[1] 28
59	60	June 4	13.12	15.40	do			do	35
61	62	June 7	12.35	12.91	do	36	29	No heads out	38
63	64	June 14	11.46	10.38	do	46	34	Heads just breaking through.	45
65	66	June 21	8.30	10.47	do	53	45	All in head	52
67	68	June 28	7.80	9.71	do	58	54	In full bloom	59
69	70	July 6	6.57	7.47	do	58	58	Seed ripened and fallen	66
71	72	July 13	21.13	18.23	Second	16	12	No sign of heads	25
73	74	Sept. 4	15.43	15.06	Third	14	15	do	52
75	76	July 17	19.56	17.88	Second			do	29
77	78	Sept. 7	15.69	16.06	Third	13	11	do	51
79	80	July 20	17.25	16.50	Second			do	32
81	82	Sept. 11	15.98	16.31	Third	12	13	do	53
83	84	July 24	16.31	15.87	Second			do	36
85	86	Sept. 14	14.69	14.38	Third	12	13	do	52
87	88	July 27	17.25	14.19	Second			do	39
89	90	Sept. 18	15.31	14.50	Third	10	10	do	53
91	92	Aug. 3	22.56	14.50	Second			do	46
93	94	Sept. 21	16.44	16.19	Third		12	do	49
95	96	Aug. 7	18.56	13.06	Second		10	do	50
97	98	Sept. 25	14.88	16.75	Third		12	do	49
99	100	Aug. 10	20.81	14.31	Second		12	do	53
101	102	Sept. 28	15.06	16.88	Third			do	49
103	104	Aug. 14	18.88	13.69	Second			do	57
105	106	Oct. 2	14.38	15.94	Third			do	49
107	108	Aug. 17	18.63	12.81	Second	22	17	do	60
109	110	Oct. 5	14.69	16.56	Third			do	49
111	112	Aug. 24	17.19	12.08	Second	22	19	do	67
113	114	Oct. 9	18.62	17.94	Third			do	46
All second cuttings:									
Average			18.86	14.83		20	14	do	
Maximum			22.56	18.23		22	19	do	
Minimum			16.31	12.08		16	10	do	
All third cuttings:									
Average			15.56	16.05		12	12	do	
Maximum			18.62	17.94		14	15	do	
Minimum			14.38	14.38		10	10	do	

[1] In computing the period up to the first cutting it has been assumed that growth began on May 1.

TABLE 6.—*Yield, protein content, and average height of reed canary grass from Marussino seed, sown June 15, 1927, in rows 36 inches apart, at Coon Creek, and mowed in 1928*

Reference No.	Date of mowing	Stage of maturity	Average height of plants	Protein content on a dry basis	Yield per acre	Length of row harvested	Remarks
			Inches	*Per cent*	*Tons*	*Feet*	
61	June 7	No heads out	36	12.35	2.7	9	First growth
63	June 14	Heads just appearing.	46	11.46	2.9	9	Do.
65	June 21	All in head	53	8.30	2.9	9	Do.
67	June 28	In full bloom	58	7.80	2.9	9	Do.
69	July 6	Seed ripened and fallen.	58	6.57	3.6	9	At end of row adjacent to fallow.
107	Aug. 17	No sign of heads	22	18.63	1.5	45	Aftermath of hay mowed June 18.

The few data on yields secured serve only to indicate what may be regarded as the maximum yields obtainable under the circumstances. (Table 6.) Only in the case of the Marussino plants was a weighing made of samples from a row in which there was a thick stand of

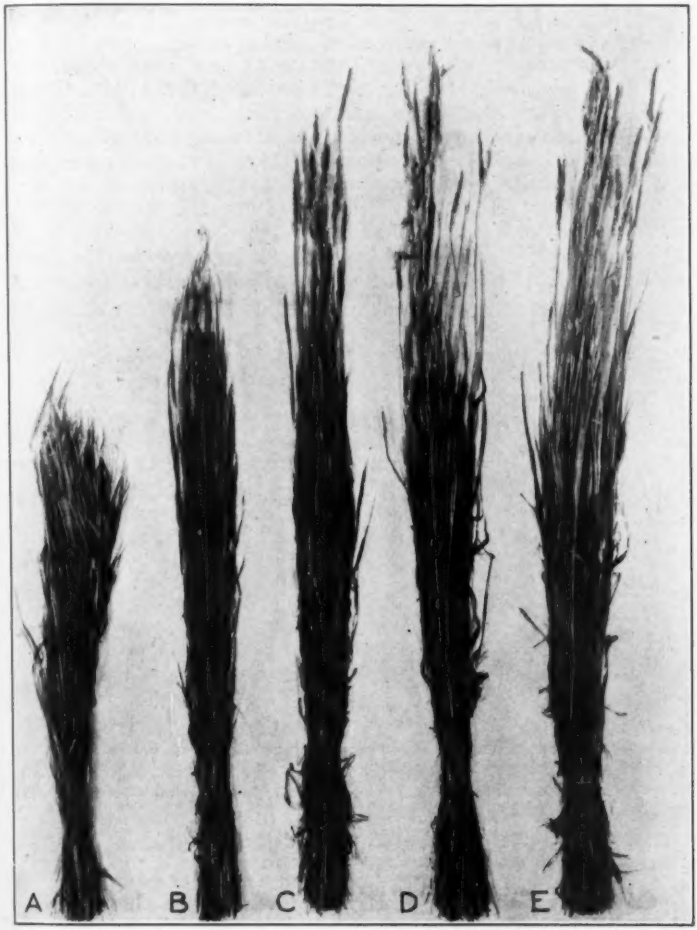

FIGURE 2.—Samples of first growth of reed canary grass sown at Coon Creek, in June, 1927, and cut on different dates in 1928. A (No. 61), Average height, 36 inches; protein content, 12.35 per cent. B (No. 63), Average height, 46 inches; protein content, 11.46 per cent. C (No. 65), Average height, 53 inches; protein content, 8.3 per cent. D (No. 67), Average height, 58 inches; protein content, 7.8 per cent. E (No. 69), Average height, 58 inches; protein content, 6.57 per cent. Numbers correspond with those in Table 6

plants, and on both sides of which there were rows which also had a thick stand. In the first five samplings of the first growth the grass from a 9-foot section of the row was weighed, but the last section, cut on July 5, being at the end of the row next to a fallow pathway,

had a larger area to draw upon for moisture and nutrients. The yield during the 3-week period, June 7 to 28, remained practically stationary. On a 45-foot row, in an equally satisfactory position for data on yields, and from which the first growth had been removed without weighing on June 18, the second cutting, on August 17, gave a yield of 1.5 tons per acre. If it be assumed that the yield of the first cutting on this was the same as that on the 9 feet of row cut on June 14, the total yield of hay from the two cuttings would amount to 4.4 tons per acre. In the computation of the yields, the rows being 3 feet apart, it has been assumed that the plants in the 9 feet of row occupied 27 square feet. The general appearance of the plants tied in bunches after sampling may be seen from Figures 2 and 3. The same scale is used in both illustrations.

GRASS FROM GERMAN SEED

At Coon Creek the first seeding was made on June 15, 1927, in rows 36 inches apart. Two days later a near-by plot was seeded broadcast

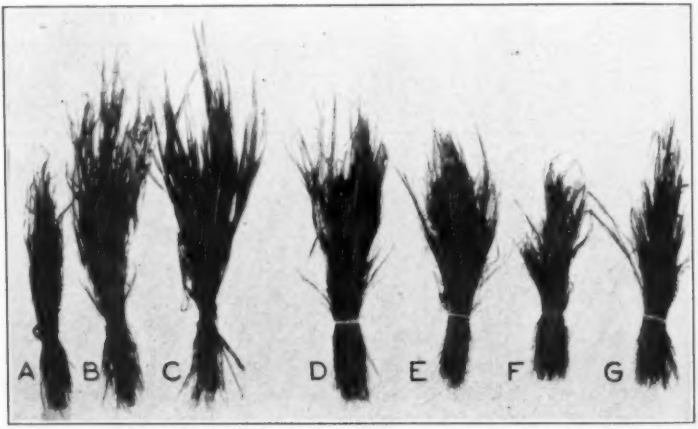

FIGURE 3.—Samples of aftermath of reed canary grass sown at Coon Creek, in June, 1927, and cut at different dates in 1928. A (No. 72), second cutting on July 13 after a growth period of 25 days; average height, 12 inches; protein content, 18.23 per cent. B (No. 108), second cutting on Aug. 17 after a growth period of 60 days; average height, 17 inches; protein content, 12.81 per cent. C (No. 112), second cutting on Aug. 24 after a growth period of 67 days; average height, 19 inches; protein content, 12.08 per cent. D (No. 73), third cutting on Sept. 4 after a growth period of 52 days; average height, 14 inches; protein content, 15.43 per cent. E (No. 81), third cutting on Sept. 11 after a growth period of 53 days; average height, 12 inches; protein content, 15.98 per cent. F (No. 89), third cutting on Sept. 18 after a growth period of 53 days; average height, 10 inches; protein content, 15.31 per cent. G (No. 98), third cutting on Sept. 25 after a growth period of 49 days; average height, 12 inches; protein content, 16.75 per cent

at the rate of 20 pounds per acre, as advised by Piper (*15, p. 231*). More than 90 per cent of the seed germinated and a very thick stand resulted. On the Page low-lime peat experimental field, 70 miles north of St. Paul, the grass was seeded broadcast on July 9, at the same rate, and an equally thick stand was obtained. About a year later, June 29, 1928, a second seeding was made on the same field but in rows 16 inches apart.

On November 4, 1927, a sample of the grass from the rows seeded on June 15 was taken and found to contain 15.25 per cent protein (No. 115). In 1928 samplings were made on the same 29 dates as the

Russian plants. (Table 5.) Up to the time of full bloom the German plants were shorter and richer in protein than the Russian. With the second cutting there was a practically steady decline from 18.23 per cent at the end of 25 days to 12.08 at the end of 67 days, although with the Russian no similar regular decline was found. The third cutting showed a higher average, 16.05 per cent, than the second and a higher minimum, 14.38, but a similar maximum 17.94. The average for the third cutting was 0.5 per cent higher than for the Russian, whereas that for the second cutting had been 4.1 per cent lower.

All the plots at both Coon Creek and Page had been fertilized liberally in 1927, and again in 1928, with 45 per cent superphosphate and 50 per cent potassium chloride. On parts of the broadcast plots at both places ammonium sulphate was applied in 1928, at Page on May 15 at the rate of 200 pounds per acre, and at Coon Creek on May 29 at the rate of 100 pounds per acre. On no part of these broadcast plots, except the borders, next to the clean cultivated roadways, did the grass make a satisfactory growth. At both places the portion given nitrogen fertilizer was taller and had a much darker color. Samples were taken from the interior of the plots at intervals in 1928, beginning at Page on June 1 and at Coon Creek on July 17 and continuing at both places up to the time of mowing the entire plots—July 21 at the former and August 7 at the latter. At neither place after the first mowing was there any appreciable growth, except along the bare roadway at Page, or any distinct effect of the ammonium sulphate application.

TABLE 7.—*Protein content, yield, and average height of reed canary grass in 1928 on plots sown broadcast with German seed in 1927.*

[Part of each plot was given an application of ammonium sulphate in 1928; at Page, 200 pounds per acre on May 15 and at Coon Creek, 100 pounds per acre on May 29]

Reference No. of samples from plots—		Date of sampling	Protein content on a dry basis		Yield of hay per acre		Average height of plants	
Without N fertilizer	With N fertilizer		Without N fertilizer	With N fertilizer	Without N fertilizer	With N fertilizer	Without N fertilizer	With N fertilizer
			Per cent	*Per cent*	*Tons*	*Tons*	*Inches*	*Inches*
AT PAGE								
116	117	June 1	10.81	15.13	0.5	0.7	(a)	(a)
118	119	June 8	10.69	13.13	.5	.7	(a)	(a)
120	121	June 19	10.84	11.25		.7	11	15
122	123	June 23	10.00	14.44	.6	.8	12	19
124	125	June 30	10.69	12.56	.6	1.2	12	19
126	127	July 7	10.88	10.63			14	24
128	129	July 15	9.81	9.56	.4	1.1	15	25
130	131	July 21	9.81	9.75	.4	1.5	16	25
AT COON CREEK								
132	133	July 17	8.75	9.38		0.9	(a)	(a)
134	135	July 20	7.94	9.56	0.8	.9	(a)	(a)
136	137	Aug. 3	8.25	7.75	.9	1.2	(a)	(a)
138	139	Aug. 7	7.63	7.63	.9	1.5	18	18

[a] No record of height.

At Coon Creek, both with and without nitrogen fertilizer, the yield and the protein content of the hay were low (Table 7), although the nitrogen application increased the former and, in the case of the earlier samplings, somewhat raised the latter. On the portion of the plot at Page given no nitrogen fertilizer the plants were short, the yield was light, and the protein content was not high at any time during the season. On the part given ammonium sulphate the growth was much taller and the yield several times as heavy. The protein content, which was 50 per cent the higher on the nitrogen-treated plot on June 1, was practically the same on both during the last three weeks.

The irregularity of the yields from the small plots at Page on successive dates, from June 8 to July 15, may be attributed to the small area, 1 square yard, harvested on each date except the last.

The scattered plants adjacent to the roadways at the ends of the plots at Page made a fairly vigorous growth after the mowing of July 21. Sufficient of these for analysis were collected at weekly intervals, beginning August 18. The protein content was found to decline gradually from 21.5 per cent on August 18 to 10.5 per cent on October 6. (Table 8.)

TABLE 8.—*Protein content and average height of reed canary grass at Page cut during latter part of the season of 1928*

[The crop from the 1927 seeding consisted of the second growth * of scattered plants from the edge of a plot sown on July 9, 1927, and given no nitrogen fertilizer; that of the 1928 seeding was from rows 16 inches apart sown on June 29, and given 200 pounds per acre of ammonium sulphate on August 17]

Reference No.		Date of sampling	Protein content on a dry basis of grass from—		Average height of plants from—	
1927 seeding	1928 seeding		1927 seeding	1928 seeding	1927 seeding	1928 seeding
			Per cent	*Per cent*	*Inches*	*Inches*
141	149	Aug. 18	21.50	23.31	14	14
142	150	Aug. 25	19.75	20.88	15	16
143	151	Sept. 1	17.25	18.81	18	19
144	152	Sept. 8	13.37	15.00	17	19
145	153	Sept. 15	12.69	14.31	17	21
146	154	Sept. 22	12.56	11.81	15	20
147	155	Sept. 29	12.19	12.44	----------	23
148	156	Oct. 6	10.50	10.38	17	24

* The first growth of these scattered plants was mowed July 21, when they were all in head and about 28 inches tall. The protein content was 10.62 per cent. (Reference No. 140.)

At Page, on June 29, 1928, Randowbruch seed was sowed in rows 16 inches apart, and seven weeks later, August 17, ammonium sulphate was applied at the rate of 200 pounds per acre. The grass, which made a very vigorous growth, was sampled at weekly intervals, beginning on August 18. It increased in height from 14 inches at the first sampling to 24 at the last, while the protein content fell from 23.31 to 10.38 per cent. (Table 8.) The yield per acre of hay, determined on October 6, amounted to 2.1 tons.

The protein content of the second growth of the scattered old plants with much fallow soil to draw upon was very similar to that of the first growth of the young plants that had been given ammonium sulphate, in both declining from over 21 per cent on August 18 to less than 11 per cent by October 6. (Table 8.)

DISTRIBUTION OF PROTEIN IN AERIAL PORTIONS OF PHALARIS ARUNDINACEA

Hosford was the first to report the protein content of leaves of *Phalaris arundiacea* separated from the rest of the plant. In his analyses he found 7.06 per cent in the leaves, 2.81 per cent in the joints, and only 0.5 per cent in the stalks without joints or leaves (*9, p. 83*). Arendt and Knop, using plants past bloom, found 16.5 per cent protein in the leaves and 4.35 per cent in the culms, including the panicles (*1, p. 50*). Feldt, referring to this paper, points out that the larger the proportion of culms in a strain of the grass the less will be its value, and that this indicates both the best time for mowing and in what direction breeding should be conducted.

Several months after the analyses reported in Tables 2 to 8 had been completed it was decided to devote some attention to the protein content of the leaves. No unground portion remained of part of the samples, but in the case of many duplicates of the original had been saved for reference as to height and stage of development. Part of these were found to have been injured to such an extent that they were unsatisfactory for analysis, but over 40 samples have been separated into either two or three parts, culms, leaves, and also panicles, wherever the last formed as much as 2 per cent of the dry samples. Where the panicle formed less it has been included with the leaves. In the case of each separate part a determination was made of the proportion which it formed of the total dry matter as well as of the protein content. (Table 9.)

With a smaller number the leaves were carefully divided into blade and sheath, and similar determinations were made. (Table 10.) For some samples data are reported in both tables. The protein content of the entire grass (Table 9) or of the entire leaf (Table 10) has been computed from the data on the different parts and is reported in the second column. Where a well-preserved specimen of the original sample was available for the separation the computed protein content has been substituted in the earlier tables for the similar, but not identical, value that had earlier been obtained by using the entire aerial portion of the plants. In such cases the same reference numbers are used. Thus, Nos. 61 to 70 refer to the same samples of grass in Table 9 that they do in Table 5. Where a sample used for a separation had suffered loss by shattering, as in the case of No. 31, or was not a part of the original samples analyzed but was simply taken from the same plot on the same day, as with No. 69–A, the letter A has been added to the original number.

TABLE 9.—*Distribution of protein and of dry matter in the aerial portion of Phalaris arundinacea grown in the crop season of 1928*

Reference No.	Protein content of grass	Percentage protein content of—			Percentage of dry matter in—			Ratio: N in leaves / N in culms	Ratio: Weight of leaves / Weight of culms	Date of sampling	Cutting	Average height of plants (inches)
		Culms	Leaves	Panicles	Culms	Leaves	Panicles					
	Per cent											
AT COON CREEK, FROM MARUSSINO SEED												
61	12.35	7.04	16.88	----------	46	54	0	2.4	1.2	June 7	First	36
63	10.38	6.23	13.58	18.95	50	41	9	2.2	.8	June 14	do	46
65	8.30	5.75	9.66	21.04	55	38	7	1.7	.7	June 21	do	53
67	7.80	4.59	11.69	15.82	60	31	9	2.5	.5	June 28	do	58
69	6.57	4.45	8.51	16.08	57	38	5	1.9	.7	July 7	do	58
69A	6.68	2.85	11.36	(1)	55	45	(1)	4.0	.8	July 7	do	58
54A	15.20	3.20	19.41	----------	26	74	0	6.1	2.8	Aug. 17	Second	16
73	15.43	6.15	18.52	----------	25	75	0	3.0	3.0	Sept. 4	Third	14
81	15.98	6.84	18.56	----------	22	78	0	2.7	3.5	Sept. 11	do	12
AT COON CREEK, FROM RANDOWBRUCH SEED												
62	12.91	7.39	14.73	30.52	29	69	2	2.0	2.4	June 7	First	29
64	11.46	5.47	14.12	21.90	37	56	7	2.6	1.5	June 14	do	34
66	10.47	5.62	12.97	17.77	41	48	11	2.3	1.2	June 21	do	45
68	9.71	5.94	13.22	14.50	49	47	4	2.2	1.0	June 28	do	54
70	6.00	3.28	11.76	9.39	50	48	2	3.6	1.0	July 6	do	58
72	18.23	6.64	20.27	----------	15	85	0	3.1	5.7	July 13	Second	12
108	12.81	3.01	15.26	----------	20	80	0	5.1	4.0	Aug. 17	do	17
112	12.08	3.08	15.94	----------	30	70	0	5.2	2.3	Aug. 24	do	19
157	17.60	7.88	19.59	----------	17	83	0	2.5	4.9	Sept. 20	Third	11
AT COON CREEK, FROM AMERICAN SEED												
13	14.82	8.34	19.32	22.98	45	43	12	2.3	1.0	June 14	First	36
14	10.56	5.20	16.49	14.41	51	41	8	3.2	.8	June 27	do	51
19	19.92	11.92	23.51	----------	31	69	0	2.0	2.2	July 21	do	23
18	15.66	6.09	20.81	----------	35	65	0	3.4	1.9	Aug. 17	Second	20

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AT PAGE, FROM RANDOWBRUCH SEED

151A	19.09	6.50	23.29	----------	25	75	0	3.6	3.0	Sept. 1	First	19
156A	13.80	5.62	15.36	----------	16	84	0	2.7	5.2	Oct. 6	....do	24

IN MADISON LAKE DISTRICT, FROM AMERICAN SEED

22	13.19	6.02	15.61	19.65	26	72	2	2.6	2.8	June 17	First	19
23	10.69	5.20	12.83	----------	28	72	0	2.5	2.6	....do	....do	24
24	14.22	7.87	19.93	17.34	45	44	11	2.5	1.0	....do	....do	36
25	14.64	7.46	17.97	10.80	31	68	1	2.4	2.2	....do	....do	29
26	12.06	5.89	15.06	12.20	32	66	2	2.5	2.1	....do	....do	28
27A	11.63	6.41	16.85	(1)	50	50	(1)	2.6	1.0	....do	....do	38
28A	13.19	6.91	17.02	28.35	49	41	10	2.5	.8	....do	....do	36
31A	8.52	4.85	13.04	19.02	56	43	1	2.7	.8	....do	....do	48
34A	11.58	5.90	16.63	(1)	47	53	(1)	2.8	1.1	....do	....do	48
37A	10.05	6.55	16.28	(1)	64	36	(1)	2.5	.6	....do	....do	48
38A	19.87	11.92	23.11	(1)	29	71	(1)	1.9	2.5	....do	....do	30
39A	19.86	8.82	22.97	(1)	22	78	(1)	2.6	3.6	July 25	Second	13

[1] Not included, part having been lost.

TABLE 10.—*Distribution of protein and of dry matter in the leaves of Phalaris arundinacea grown in the crop season of 1928*

Reference No.	Percentage protein content of—			Percentage of dry matter of leaf in—		Ratio: N in blade / N in sheath	Ratio: Weight of blade / Weight of sheath	Date of sampling	Cutting	Average height of plants (inches)
	Entire leaf	Sheath	Blade	Sheath	Blade					
61A	15.04	7.50	20.07	40	60	2.7	1.5	June 7	First	36
27A	16.85	8.44	22.70	41	59	2.7	1.4	June 17	....do	38
37A	16.28	9.12	22.13	45	55	2.4	1.2	....do	....do	48
14A	15.73	9.46	21.29	47	53	2.2	1.1	June 27	....do	51
69A	11.36	6.03	15.55	44	56	2.6	1.3	July 7	....do	58
151A	23.29	13.19	26.31	23	77	2.0	3.3	Sept. 1	....do	19
156A	14.75	8.12	17.87	32	68	2.2	2.1	Oct. 6	....do	24
158	17.21	6.90	20.47	24	76	3.0	3.2	Aug. 10	Second	
54A	19.41	8.51	23.04	25	75	2.7	3.0	Aug. 17	....do	16
143A	23.91	12.81	26.87	21	79	2.1	3.8	Sept. 1	....do	18
148A	15.36	6.88	17.50	20	80	2.5	4.0	Oct. 6	....do	17
157	19.64	9.73	21.96	19	81	2.3	4.3	Sept. 20	Third	

In all cases the percentage of protein in the leaves was much larger than in the culms, the ratio for all averaging 2.8. The proportion of the dry weight formed by the leaves varied in the first cutting from 31 to 72 per cent and in the second and third cuttings from 65 to 85 per cent. With the first cutting the proportion fell up to the time of bloom, in the case of the Marussino grass declining from 54 per cent on June 7 to 31 per cent three weeks later. (Nos. 61 and 67 in Table 9.) On each of the different first dates of cutting the Randowbruch grass showed a higher proportion of leaves than the Marussino. The panicles were in most cases richer in protein than the leaves and in all cases richer than the culms, but in none of the samples did they constitute as much as 12 per cent of the whole. The decrease in the proportion of leaves in the grass up to the time of bloom that accompanied advancing maturity of the first growth was not paralleled by as large a decrease in their richness in protein, as may be seen from the fourth column of Table 9.

Within the leaf itself there was a marked difference between the blade and the sheath. The blade formed from 53 to 81 per cent of the dry matter of the leaf. In the samples reported in Table 10 the percentage of protein in the blades is from 2 to 2.7 times as high as in the sheaths. Thus, in general, the blade was found at least twice as rich in protein as the sheath and the entire leaf was more than twice as rich as the culm. Accordingly, the higher the proportion of culm and sheath the lower would be the percentage of protein. As the length of the sheath increases along with that of the culm, the percentage of protein in the hay may be increased both by mowing before the culms and sheaths have reached their full development and by the selection and breeding of strains of the grass that are characterized by short internodes.

PROTEIN CONTENT OF PHALARIS ARUNDINACEA COMPARED WITH THAT OF TIMOTHY

Timothy is at present the most important grass for meadows on northern peat soils, being sown either with alsike and medium red clovers or with alsike alone. For two or three years, and sometimes longer, this practice gives a hay rich in clover, but later the clovers disappear, leaving timothy as a pure stand or in mixture with various volunteer grasses. In order to justify its substitution for timothy on land that is sufficiently well drained, reed canary grass must be shown to be capable of furnishing more protein per acre than the average from a timothy-clover mixture for the first four or five years after seeding.

At both Page and Coon Creek some comparisons were made of the protein content of the timothy with that of reed canary grass. At Page, on a plot near that on which reed canary grass had been sown broadcast in the summer of 1927, timothy was sown at the rate of 12 pounds per acre without admixture with any other seed, and a full stand was secured. In 1928 part of this was given 200 pounds per acre of ammonium sulphate at the same time that this fertilizer was applied to part of the plot of reed canary grass. Beginning on June 1, samples for analysis were taken at approximately weekly intervals from the two parts of both plots. (Table 11.)

TABLE 11.—*Comparison of protein content and yield of reed canary grass and timothy grown with and without nitrogen fertilizer on Page peat experimental field in 1928*

[Both grasses sown broadcast in July 1927]

PROTEIN CONTENT OF MOISTURE-FREE GRASS

Date of sampling or mowing	Without N fertilizer		With N fertilizer	
	Reed canary grass	Timothy	Reed canary grass	Timothy
	Per cent	*Per cent*	*Per cent*	*Per cent*
June 1	10.81	13.75	15.13	15.07
June 8	10.69	13.75	13.13	14.56
June 23	10.00	13.31	14.44	11.94
June 30	10.69	11.62	12.56	9.96
July 7	10.88	9.37	10.63	8.75
July 15	9.81	10.87	9.56	7.31
July 21	9.81	9.00	9.75	6.56
Average	10.38	11.67	12.17	10.59

YIELD PER ACRE OF HAY

July 21	*Ton* 0.4	*Ton* 1.0	*Tons* 1.5	*Tons* 1.5

YIELD PER ACRE OF PROTEIN

July 21	*Pounds* 66	*Pounds* 153	*Pounds* 248	*Pounds* 170

On the subplots given no nitrogen fertilizer the timothy was the richer in the four samplings in June, but the poorer in two of the three in July. On the plots given ammonium sulphate the timothy was much the poorer in protein in all the samplings except the first and second. The yield of air-dry hay, mowed on July 21, was alike on the nitrogen-treated portions—1.5 tons per acre—but on the portions given no nitrogen fertilizer there was 1 ton of timothy as compared with only 0.4 ton of reed canary grass. By comparing the yields of protein per acre from the four subplots it will be seen that it was highest on the reed canary grass given nitrogen and lowest on the same grass given none.

At Coon Creek similar plots of the two grasses were sown broadcast early in the summer of 1927. (Table 12.) The timothy used for comparison received no nitrogen fertilizer but made the more rapid growth and the first cutting had been removed before sampling of the reed canary grass was begun. The hay from the latter, mowed on August 7, gave a lower yield but had a somewhat higher protein content than the timothy mowed on July 10.

TABLE 12.—*Comparison of protein content and yield of reed canary grass and timothy grown without nitrogen fertilizer on Coon Creek peat experimental field in 1928*

[Both grasses sown broadcast in summer of 1927. Timothy mowed July 10, but reed canary grass not until August 7]

PROTEIN CONTENT OF MOISTURE-FREE GRASS

Date of sampling or mowing	Timothy	Reed canary grass
	Per cent	*Per cent*
June 13	11.52	
June 27	9.77	
June 30	8.02	
July 10	6.21	
July 17		7.44
July 30		6.74
Aug. 3		7.01
Aug. 7		6.49

YIELD PER ACRE OF HAY

	Tons	*Tons*
August 7	1.3	0.9

YIELD PER ACRE OF PROTEIN

	Pounds	*Pounds*
August 7	144	95

DISCUSSION OF YIELDS OF DRY MATTER AND PROTEIN

The yields of reed canary grass hay obtained from the broadcast seedings at both Coon Creek and Page were very poor. Where no nitrogen fertilizer was applied the yield for the entire season was less than a ton per acre, and only 1.5 tons where this was used. The applications of phosphate and potash had been liberal, the supply of lime was adequate at both places, naturally so at Coon Creek, and as the result of a liberal marling at Page. At Page the temperatures had been favorable and the rainfall unusually plentiful, but at Coon Creek the rainfall was about 20 per cent below the normal. The distance of the water table below the surface at Coon Creek was almost stationary throughout the season, and at Page it did not vary widely until near the close of the season. (Table 13.) At both places a higher average level would have been more favorable for grasses in general and probably especially so for *Phalaris arundinacea*, but the level that prevailed was very favorable for the nitrification of the peat. In general, the conditions at both places were so favorable for grass that the low yield of hay from the broadcast seeding should not be attributed to unusual weather. It was more probably due to an insufficiency of available nitrogen.

TABLE 13.—*Water level, rainfall, and mean temperature at Coon Creek, Page, and in the Lake Madison district during the crop season of 1928*

EXTREMES OF DISTANCE OF WATER TABLE BELOW SURFACE, IN INCHES

Locality, crop, and manner of sowing	May	June	July	August	September
Coon Creek, German grass broadcast		25–34	24–37	21–35	26–38
Coon Creek, Russian and German grass, in rows		27–42	27–39	36–43	30–41
Coon Creek, American grass in rows		43–46	46–53	46–54	45–53
Page, German grass broadcast	27–33	23–34	20–29	13–30	10–24
Page, German grass in rows	27–30	15–31	10–22	13–22	6–20

PRECIPITATION, IN INCHES

Coon Creek	2.2	2.3	3.1	4.8	3.6
Page	.8	4.2	6.3	5.5	3.2
Madison Lake [1]	2.0	2.9	2.0	8.8	1.9

MEAN TEMPERATURE, 0° F.

Coon Creek	61	63	72	70	59
Page	58	60	69	67	55
Madison [1]	62	63	73	70	59

[1] From record at St. Peter, 12 miles from Lake Madison.

The yield of grass sown in rows 3 feet apart was very good. The Marussino strain at Coon Creek, sown at practically the same time as the near-by broadcast plots of German grass and given the same fertilization, produced nearly three times as much hay as the latter. The first cutting on June 18, with a yield of 2.9 tons per acre, may be assumed to have had the same composition as No. 65, sampled three days later (Table 5), and so to have contained 481 pounds of protein; while the second, mowed on August 17, with 18.6 per cent protein (No. 107), gave a yield of 1.5 tons of hay and so of 558 pounds of protein per acre. A yield of 4.4 tons per acre of hay from a meadow is to be considered high and a yield of 1,039 pounds per acre of protein exceptionally high. The German and American grass in rows grew equally well, but data indicating the yields of these on a field scale were not secured.

There is much difference of opinion as to the suitability of peat soils for *Phalaris arundinacea*. Werner in 1889 stated that although it is especially productive on moist soils it does not succeed on peat (*25, p. 117*), and Strecker as late as 1914 mentions that it does not love peat soils (*23, p. 179*). Piper's statement that "the grass is said not to thrive in peaty soils" (*15, p. 231*) may be based on the opinions of these two authors. In 1892 Von Seelhorst recommended it as a valuable grass on very wet peat meadows, especially on those exposed to flooding, but emphasized the fact that in order to secure hay of good quality the grass should be mowed "before blooming at the end of June" and recommended it only for those meadows that are too wet for the commonly used grasses, such as timothy and meadow fescue (*18, p. 137*). Bersch in 1912 took the same view (*3, p. 251*).

Heuser, of Danzig, in 1927 states that it is only within the last few years that it has come to be recognized that *Phalaris arundinacea* is one of the most valuable grasses for the peat lands, mentioning that it is especially exacting in its demands for nitrogen (*11, p. 139*).

Weber considers the character of the soil to be of little importance for the growth of this grass provided the moisture supply and its oxygen content are favorable and there is an abundance of available plant nutrients, either naturally present, supplied in the water, or applied as fertilizers. It grows on sand, loam, marshy clay, peaty soil, high-lime peat (Niedermoor), low-lime peat (Hochmoor) to which lime has been applied, on moderately acid to neutral soils, and on chalk (*24, p. 16*).

SUMMARY AND CONCLUSIONS

Previous determinations of the crude protein content of *Phalaris arundinacea*, about 30 in number, covering a period of 80 years, and representing 5 European countries and 6 different States in this country, show a great range, with a maximum of 22 per cent and a minimum of 3.44 per cent of the moisture-free grass.

Protein determinations are reported on upward of 150 samples of the grass grown on peat soils in Minneosta, using plants from Russian and German as well as American seed. In the case of more than 30 of these samples the plants were separated into leaf, culm, and panicle, the weight and protein content of each being determined, while with 12 samples the leaves were divided into blade and sheath for analysis.

The highest percentage of protein found in the entire grass, on a moisture-free basis, was 25.2 and the lowest 6.6. It ranged in the culms from 2.8 to 11.9 per cent, in the leaves from 8.5 to 23.5, and in the panicles from 9.4 to 30.5. The maximum found in the culms was higher than the minimum in the leaves, but in the case of every sample there was a much higher percentage in the leaves than in the culms, generally from two to three times as much. The leaves formed from 31 to 72 per cent of the dry matter in the first cutting and from 65 to 85 per cent in the aftermath. With advancing maturity of the first growth up to the time of bloom there was a rapid fall in the proportion of the total dry matter formed by the leaves without a corresponding decline in their protein content.

In the panicles, which formed less than 12 per cent of the dry weight of the first growth and were absent from the aftermath, the protein was usually higher than in the leaves and varied independently of the latter. The blade formed from 53 to 81 per cent of the dry matter of the leaf and its protein content varied from 15.5 to 26.87 per cent, while that of the sheath varied from 6 to 13.2 per cent. The amount in the sheath was only from one-third to one-half as high as that in the blade and the two values rose and fell together.

A higher protein content was favored by early mowing, thin stands, and an increased supply of available nitrogen. In the aftermath the protein was less variable and in general much higher than in the first growth. Differences in the protein content of grasses of different strains, sown at the same time and harvested at the same stage of maturity, were at most very small compared with differences connected with the degree of maturity and supply of nitrogen.

The grass seeded broadcast gave a much lower yield of hay and a very much lower yield of protein than where it was sown in rows on adjacent similar land, similarly fertilized and the weeds and other grasses kept down until it became established. The yield and protein content of the hay from *Phalaris arundinacea* appears unusually

sensitive to the supply of available nitrogen in the soil, and the protein content is very much influenced by the stage of maturity and consequent proportion of culm and sheath.

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THE MORPHOLOGY AND BIOLOGY OF EULIMNERIA CRASSIFEMUR, AN IMPORTANT PARASITE OF THE EUROPEAN CORN BORER [1]

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INTRODUCTION

Eulimneria crassifemur (Thomson) is an important parasite of the larva of *Pyrausta nubilalis* Hbn. in Europe and, with other parasites, has been studied by the staff of the European parasite laboratory at Auch and Hyères, France, since 1919. Shipments of this species to the United States have been made in varying quantities each year since that date. It is therefore deemed advisable to publish such information as is now possessed concerning its biology and habits in order to aid various scientific workers engaged in manipulating and colonizing this parasite.

SYSTEMATIC POSITION AND SYNONYMY

This parasite is a species of the genus Eulimneria, the tribe Campoplegini, subfamily Ophioninae, family Ichenumonidae, and order Hymenoptera. It was first described by Thomson (*13, p. 1106*)[4] as a species of Holmgren's genus Limneria. This name was changed to Limnerium by Ashmead in 1900 (*1*). In 1907 the genus Eulimneria was erected by Schmiedeknecht (*7*) for the group of species including *crassifemur*. Szépligeti (*12, p. 16*), however, lists it under Limnerium in a fascicle of the Genera Insectorum published in 1911. Smits van Burgst (*10, p. 144*) considers this species identical with *Campoplex lineolatus* Bouché, a parasite of (*Tinea*) *Hyponomeuta evonymella* L., a European apple pest (Lepidoptera).

GEOGRAPHICAL DISTRIBUTION

Originally described from Sweden and cited by Dalla Torre (*4, p. 93*) in his Catalogus Hymenopterorum as occurring there, this species is listed in Genera Insectorum as occurring in Sweden and Germany. It occurs also in Belgium, France, Italy, Yugoslavia, Hungary, Switzerland, and Spain. In France the parasite has been found in the following localities: The southwestern plain, extending up into the valleys of the Pyrenees; the Rhône Valley around Valence (but not south of Avignon); the valley of the Main around Angers; the basin of the Seine around Paris; the region of Lille; the Rhine Valley around Strasbourg and Colmar; and the region of the Jura near Sellières. This species has never been taken at Hyères or at any other point in the French Mediterranean zone. In Belgium it occurs at Brussels and Antwerp. In Italy it has been taken at Pavia, Bergamo, and Piacenza in the Po Valley, as well as at Udine and Naples. In Spain it has been found in the Province of Galicia.

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[2] Resigned June 1, 1928.
[3] The authors acknowledge their indebtedness to Mrs. W. R. Thompson for her assistance in the preparation of many of the illustrations.
[4] Reference is made by number (*italic*) to "Literature cited," p. 344.

HOST RELATIONS

Eulimneria crassifemur has been reared by Schwangart (*8, p. 555*) as a parasite of the larvae of (*Conchylis*) *Clysia ambiguella* Hbn., the grapevine conchylis, and (*Eudemis*) *Polychrosis botrana* Schiff., the European grape berry moth, in Austria. Trägårdh (*19, p. 420*) reared it from *Lyda signata* Fab., the spruce sawfly, while Smits van Burgst (*10, p. 144*) reports it from *Evetria buoliana* Schiff., the European pine shoot moth. The writers are of the opinion that the *E. crassifemur* reared from *Pyrausta nubilalis* may not be identical with the species reared from the above-mentioned hosts.

Paillot (*5, p. 190*) has reared a species which he calls *Eulimneria crassifemur* from (*Neurotoma*) *Lyda nemoralis* L., the peach sawfly,

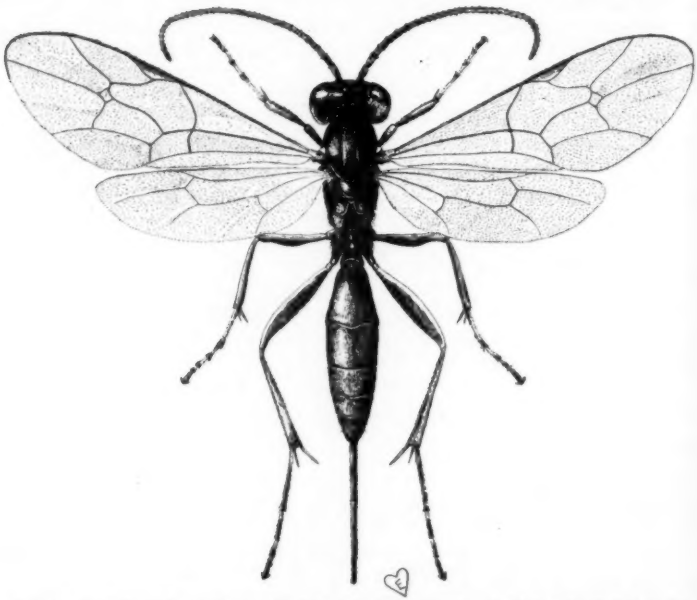

FIGURE 1.—*Eulimneria crassifemur*, adult female. The second and third abdominal segments appear considerably foreshortened in this drawing owing to the curvature of the body

but, according to this author, his species differs from the parasite of *Pyrausta nubilalis* in that it has a shorter ovipositor.

The writers of the present paper have reared *Eulimneria crassifemur* from *Pyrausta nubilalis*, collected from all the localities mentioned in Spain, France, Belgium, and Italy, and from south Wurttemberg in Germany, while K. W. Babcock and A. N. Vance have reared it in Hungary and Yugoslavia.

DESCRIPTION OF THE ADULT

Eulimneria crassifemur (fig. 1) is a black-bodied, red-legged, wasplike insect about one-half inch long, with a protruding ovipositor. It differs from the other campoplegines parasitic on *Py-*

rausta nubilalis in having an areolet in the forewings, no yellow or red on the body, middle and hind coxae and hind trochanters black, and tips of hind tibiae and hind tarsi black, but proximal extremities of first three tarsal joints dirty white. The ovipositor is about one-half the length of the abdomen. This species is more likely to be confused with *Dioctes punctoria* Roman than with any other parasite of the corn borer, but it can be readily separated from that species by the presence of the areolet.

DESCRIPTION OF IMMATURE STAGES

EGG

The egg (fig. 2, A) averages 2.9 mm. long and 0.9 mm. wide. It is oblong ovate, slightly flattened on one side, and entirely without spines, sculpturing, or processes of any kind. When first deposited it is white, but within a few days it turns a dark brown and can then be distinguished through the skin of the host larva.

PRIMARY LARVA

The first-stage larva (fig. 3, A) is of the typical ichneumoniform type. Cylindrical, without spines, tubercles, or segmentary appendages, composed of a heavily chitinized head followed by 13 body segments; the last or tenth abdominal segment prolonged ventrally into a conical, pointed tail about one-half as long as the rest of the body.

Head thimble-shaped, slightly pointed anteriorly, and of a light brown color; dorsally from the posterior border two thinly chitinized grooves extend forward over three-fourths of the length of the head; laterally (fig. 2, H) with an irregular series of small circular sensorial organs; dorsally (fig. 2, D) one pair of large sensorial hairs; posteriorly and slightly above the lateral line another pair, while on the ventral portion there are four such pairs. Mouth parts distinctly delineated. The labrum with seven pairs of sensorial organs, the labial region with three, and the maxillary region with three. (Fig. 2, B.) Mandibles simple, strongly curved, and rather sharply pointed.

Body cylindrical, tapering but slightly posteriorly, segments about equal in length. The cuticle bears no tubercles or spines and no visible sensorial organs.

Digestive system well developed and the fore, middle, and hind intestines readily distinguished as well as the Malpighian tubes and the branched salivary glands, which extend the entire length of the mid intestine. Brain and ventral nerve cord prominent, the heart distinguishable by its more or less regular pulsations at intervals of three to four seconds. The fat body but slightly developed, and the only part of it that can be readily distinguished is in the tail (figs. 2, E and 3, B); small fibers which are probably hypodermal attach this lobe of the fat body to the hypodermal cells of the tail.

The tracheal system (fig. 3, B) is as follows: A trunk runs along each side of the body from the first thoracic to the ninth abdominal segment; these trunks are joined anteriorly by a transverse commissure which passes above the digestive tract in the first thoracic segment; posteriorly they are joined in a like manner by a ventral commissure in the anterior portion of the ninth abdominal segment. A secondary lateral trunk extends from the posterior margin of the first thoracic to the posterior margin of the third thoracic segment.

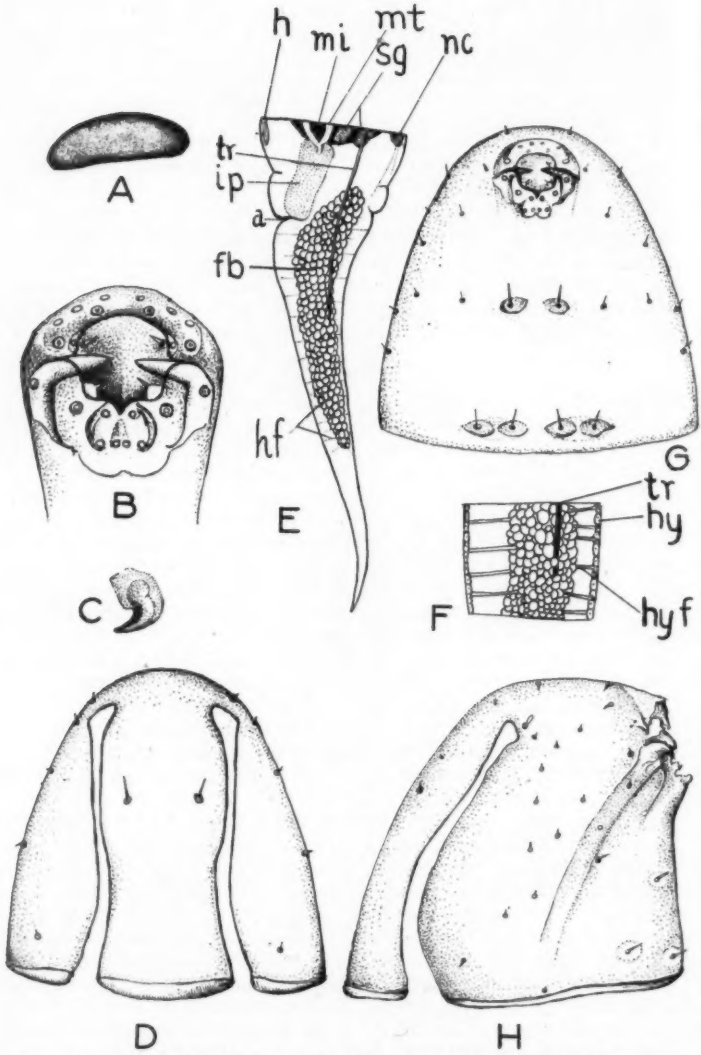

FIGURE 2.—*Eulimneria crassifemur*: A, egg, several days old; B to H, larval details; B, mouth parts of stage 1; C, mandible of stage 2; D, dorsal view of head skeleton of stage 1; E, caudal extremity of stage 1 showing fat body (*fb*), trachea (*tr*), hypodermal fibers (*hf*), nerve cord (*nc*), hind intestine (*ip*), midintestine (*mi*), heart (*h*), Malpighian tubes (*mt*), salivary glands (*sg*), and anus (*a*); F, portion of caudal extremity enlarged, section about middle of the fat body, showing trachea (*tr*) and its connection with the fat body, hypodermis (*hy*), and hypodermal fibers (*hyf*); G, ventral view of head of stage 1; H, lateral view of head skeleton of stage 1

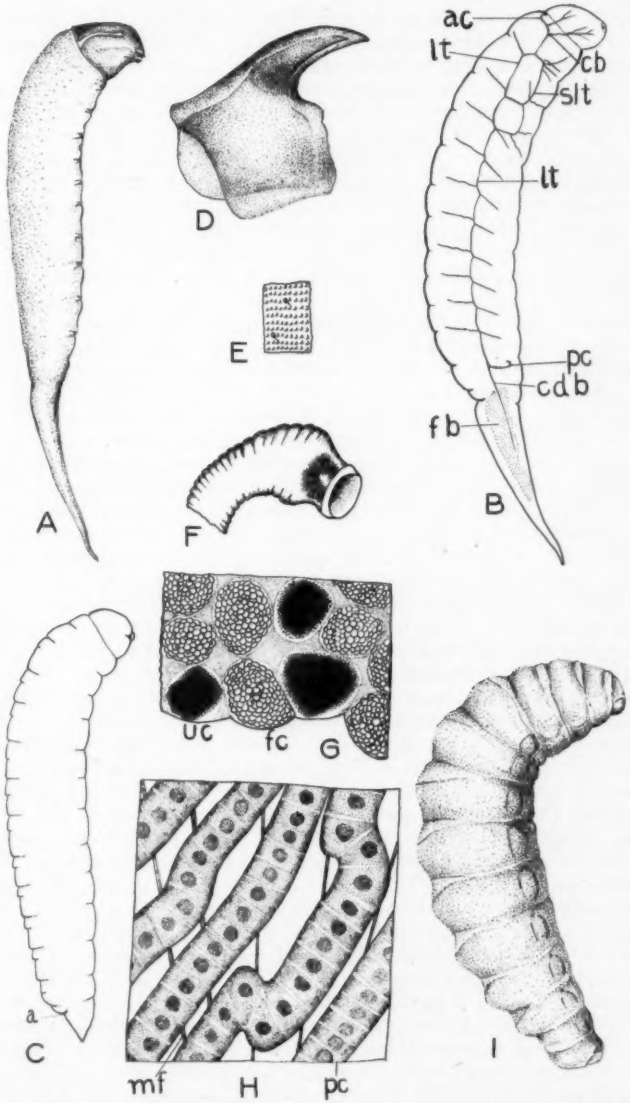

FIGURE 3.—Larval details of *Eulimneria crassifemur:* A, First-stage larva before feeding; B, first-stage larva after feeding, showing tracheal system, fat body (*fb*) in caudal process, anterior commissure (*ac*), posterior commissure (*pc*), lateral trunk (*lt*), secondary lateral trunk (*slt*), cephalic branch (*cb*), and caudal branch (*cdb*); C, side view of stage 2 larva, (*a*) anus; D, mandible of full-grown larva; E, portion of skin of full-grown larva showing microscopic tubercles which entirely cover the body and two of the sensorial papillae; F, spiracle of full-grown larva; G, portion of fat body near anterior end of stomach where the urate cells appear, seen by transmitted light, fat cells (*fc*), urate cells (*uc*), H, small portion of pericardial membrane showing pericardial cells (*pc*) arranged in irregular tubules; and dilator muscle fibers (*mf*); I, side view of full-grown larva

This trunk is attached to the principal lateral trunk by three dorsoventral commissures located in the first to third thoracic segments; this secondary lateral thoracic trunk, which Seurat appears to have been the first to notice, seems to occur throughout the Ichneumonidae and is probably characteristic of that group. The anterior transverse commissure gives off branches to the head, the main lateral trunk furnishes a dorsal and a ventral branch to each segment, the secondary lateral trunk furnishes ventral and lateral branches to the three thoracic segments, and a branch arising at the posterior end of the main lateral trunk continues into the tail, where it becomes lost among the fat cells which fill the lumen of that organ. The lumen of the tail is continuous with that of the body. There are no open spiracles.

The tail, which exists in various forms in a considerable variety of parasitic larvae (Ichneumonidae, Braconidae, Chalcididae), has been the subject of a good deal of rather fruitless speculation. Seurat (*9, p. 90*) considers it to be a locomotory organ, Timberlake (*17, p. 85*) and others to be a blood gill, Weissenberg (according to Tothill) believes it to be used for storing excretory substances.

Tothill (*18, p. 71*) considers the tail to be a respiratory organ. According to his description of the larva of *Campoplex pilosulus* Prov., it is anatomically a special sort of gill and is composed of a mass of small tracheal tubes each of which terminates in a large vesicle lying in the "wall" of the gill, which is thus lined with numerous large air cavities separated by narrow protoplasmic walls. The writers have identified without difficulty in the larva of *Eulimneria crassifemur* all the structures represented by Tothill in his figures of the larva of *C. pilosulus*. They are convinced, however, that Tothill's interpretation of his preparations is erroneous. The mass of material represented within the cavity of the tail in Tothill's Figure 55, and said by him to be a bundle of tracheids, is in reality simply a lobe of adipose tissue from which the fat globules have been dissolved out by the reagents employed, so that the appearance in section is similar to that of a mass of tubules. The so-called intracellular air spaces into which the tracheids are said to open in the wall of the tail are simply extensions of the body cavity incompletely separated by protoplasmic strands running from the hypodermis of the tail to the adipose tissue lying within it. The true structure of the tail is easily determined by the examination of living larvae, in which it can be seen that the only respiratory structures of the organ are two fine tracheae which pass backward into the lobe of adipose tissue; that the so-called intracellular air spaces contain no air, but are filled with liquid; and, finally, that the central mass described by Tothill is a lobe of the fat body. The tail is therefore not anatomically a tracheal gill. None of the hypotheses which have been advanced in regard to its function appear to the writers to be satisfactory, and they are inclined to doubt that this organ, in spite of its anatomical prominence in certain parasitic larvae, is physiologically of any particular importance.

SECONDARY LARVA

The secondary larva (fig. 3, C), as is generally the case in this type of hymenopterous larvae, differs from the primary larva principally by its large size, its soft and more hemispherical head, and its shrunken tail process. There are no open spiracles in this stage.

MATURE LARVA

Mature larva (fig. 3, I) 10 mm. long and 3 mm. wide, subcylindrical, tapering slightly anteriorly and posteriorly. General color a dirty white, with the snow-white urate cells showing through the skin. Cuticle with tiny tubercles thinly spaced, visible only under the microscope. Head hemispherical but slightly flattened dorsoventrally; with numerous sensorial organs around the mouth parts[5] and on its upper surface a pair of brownish disks which correspond to the antennae. The various appendages of the buccal region distinctly represented. Each of the thoracic segments bears ventrally a pair of light-brown disks representing the legs, while the second and third segments bear a lateral pair of similar disks representing the wings. Each segment has a number of tiny hairlike sensoria, the number and arrangement of which are shown approximately in the diagram in Figure 4, C. The eighth and ninth abdominal segments each bear a pair of light-brown disks in the female larva, whereas in the male larva there is only a single pair located on the posterior edge of the ninth abdominal segment. These disks surround the pedicels of the histoblasts of the external reproductive organs. The posterior lobe, which represents the remains of the tail process, terminates in a small cuticular spike.

The tracheal system (fig. 5, A) is essentially the same as in the primary larva; the branches are, however, considerably more ramified and there are now nine pairs of open spiracles located on the first thoracic and the first eight abdominal segments.

The integumentary muscular system (fig. 4, A) consists of a longitudinal dorsolateral group (dl1–dl6), a ventrolateral group (vl1–vl6), a series of diagonal muscles extending anteriorly in a dorsoventral direction (a1–a4), a series of diagonal muscles extending anteriorly in a ventrodorsal direction (b1–b3), and a series of small dorsoventral muscles (t1–t5) of which four occur only in abdominal segments 1 to 9, inclusive. The diagonal muscles b1 and b2 and a1 do not traverse the entire length of the segment but are attached to the integument at a point somewhat behind the forward intersegmental line. It is this attachment which gives the double segmental appearance to the Eulimneria when seen in profile. In the ninth abdominal segment the muscle dl3 extends backward across the tenth segment and attaches to the rectum, and there seems to be a supplementary diagonal muscle (a5) in about the same position as a2 but external to the transverse muscle (t2–5). The integumental musculature is thus of a very simple type.

The cephalic musculature is composed principally of four groups, the first of which extends from the labrum to the wall of the head immediately above it, the second group extends in like manner from the esophagus upward to the chitin of the head, the third group extends from near the back of the head forward to the region around the mouth, while the fourth group extends from the periphery of the head near the posterior lateral border to the region of the labium. The labial and esophageal groups are active principally during the process of ingestion.

The fat body (fig. 3, G) consists principally of four more or less regular lobes surrounding the digestive tract, placed internally to the

[5] The arrangement of these organs is constant and will, the writers are convinced, prove of considerable value for purposes of identification; it seems to be identical in *Eulimneria crassifemur* and *Dioctes punctoria* Roman.

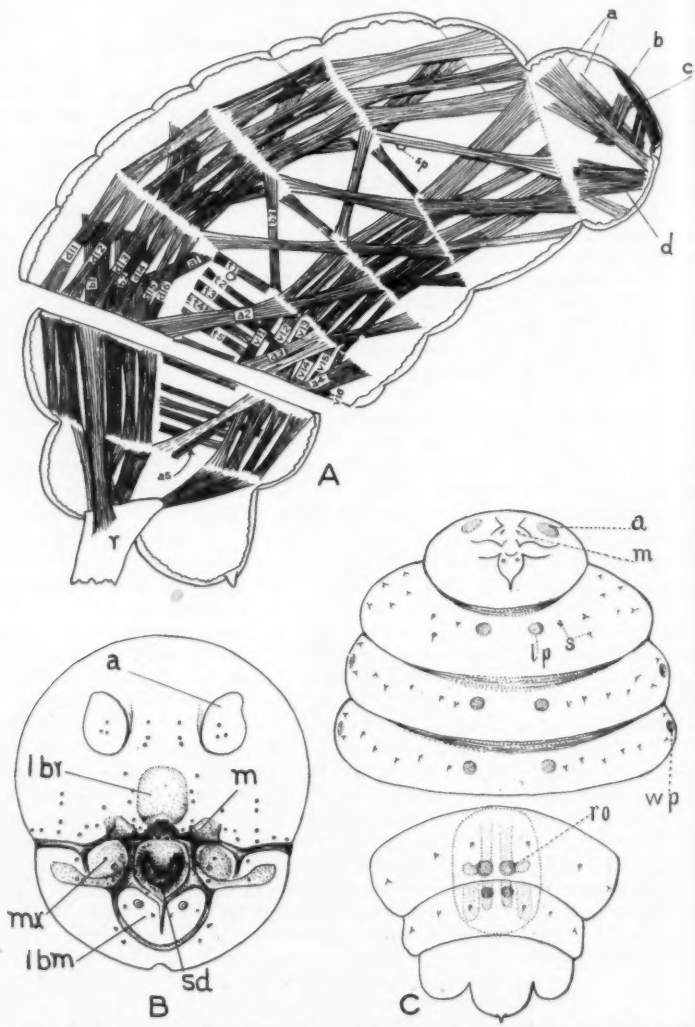

FIGURE 4.—Larval details of *Eulimneria crassifemur:* A, Integumentary muscles of full-grown larva (left side) showing the head, the three thoracic, and the first, ninth, and tenth abdominal segments, dorsolateral group (*dl*), ventrolateral group (*vl*), anterodorsoventral diagonal muscles (*a*), anteroventrodorsal diagonal muscles (*b*) dorsoventral transverse muscles (*t*), spiracle (*sp*), and rectum (*r*); B, head of full-grown larva (front view), antenna (*a*), labrum (*lbr*), mandible (*m*), maxilla (*mx*), labium (*lbm*), salivary duct (*sd*); C, drawing of head, thorax, and eighth to tenth abdominal segments (from below) of full-grown female larva to show external organs, sensoria, etc., leg pads (*lp*), wing pads (*wp*), reproductive organs (*ro*), sensoria (*s*), antenna (*a*), and mandible (*m*)

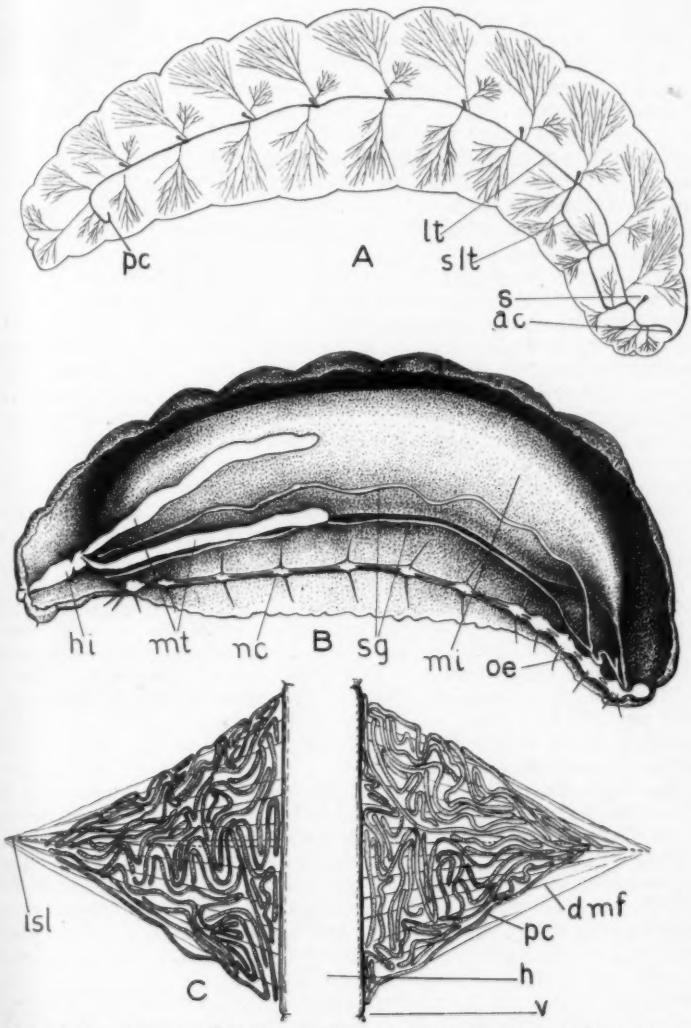

FIGURE 5.—Larval organs of *Eulimneria crassifemur:* A, Respiratory system of full-grown larva, spiracle (*s*), lateral trunk (*lt*), secondary lateral trunk (*slt*), anterior commissure (*ac*), posterior commissure (*pc*); B, internal organs, esophagus (*oe*), mid intestine or stomach (*mi*), hind intestine (*hi*), salivary gland (*sg*), Malpighian tubes (*m*), nerve cord (*nc*); C, segment of heart showing pericardial cells (*pc*), dilator muscle fibers (*dmf*), valve (*v*), and intersegmental line (*isl*)

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integumentary muscles, while in each segment between the skin and the integumentary muscles there is a thin layer of adipose tissue. Miscellaneous conglomerations of minor importance exist at several other points in the anterior and posterior ends of the larva. The fat body is grayish white and the cells are quite distinct under magnification; the lobes which surround the mid intestine are thickly interspersed with urate cells wherein the accumulated granules are seen as snow-white lumps distributed among the fat. It is remarked that these urate cells occur only in the fat which is in contact with the mid intestine; the anterior and posterior ends of these fat lobes do not bear any urate cells nor do the lobes external to the integumentary muscles or the miscellaneous lobes. Figure 3, G, shows the fat cells as seen by transmitted light in tissue taken from the anterior extremity of the urate cell region.

The digestive tract (fig. 5, B) consists of the short, almost straight esophagus, fore intestine, mid intestine, or enlarged stomach without a posterior opening, and the hind intestine. The hind intestine is composed of two enlarged portions and a narrow rectum. The enlarged portions are separated by a narrow constriction in which there is a valve. The salivary glands consist of four slender tubes extending along the entire length of the mid intestine, the two tubes of each side uniting in the thorax and opening into the mouth by a very short common duct. Tothill (*18, p. 66, fig. 41*) describes in *Campoplex pilosulus* a "cut-off" duct leading from the salivary gland duct to the esophagus. The writers have not observed any such communication between these ducts in Eulimneria and are inclined to doubt its existence.

The Malpighian tubes arise from the hind intestine and extend forward almost one-half the length of the stomach; they are four in number and are larger than the salivary glands.

The nerve cord (fig. 5, B, *nc*) consists of the supraesophageal and subesophageal ganglia and 11 additional paired ganglia, the last of which probably represents three paired ganglia united as it gives off three pairs of branches. Tothill (*18*) shows 13 distinct and widely separated pairs of ganglia behind the subesophageal ganglia in his figures of *Campoplex pilosulus*.

The heart or dorsal vessel (figs. 3, H, and 5, C) is a narrow straight tube with the usual chambers and valves as found in hymenopterous larvae. It originates in the ninth abdominal segment and extends forward to the brain. It is bordered on each side from the ninth to the second abdominal segments by fan-shaped series of pericardial cells (figs. 3, H, and 5, C) which lie ventrally to a series of fine muscular fibers attaching the heart walls to the integument. The heart beats more or less regularly every three to four seconds with occasional pauses. With each pulsation the brain moves slightly.

PREPUPA

The prepupa is very much like the larva except that it is less arched dorsally, slightly more pointed anteriorly, and somewhat more yellowish in color. The red eyes can be seen beneath the old larval skin as can the other imaginal disks.

COCOON

The winter cocoon (fig. 6) varies from light gray to almost black. It is oblong oval, of solid texture; the lighter colored specimens exhibit

a faint whitish ring around the middle, but in the dark specimens no such ring is visible. The summer cocoons, which have been found only in northern Italy, are often thin and whitish with a distinct, paler ring around the middle. The summer generation in that region spins a mixture of the thick and thin types of cocoons from both of which the adults emerge before the winter sets in.

BIOLOGY

HABITS OF THE ADULT

The adult, having shed the old pupal skin, chews an irregular hole about 2 mm. in diameter slightly to one side of the end of the cocoon and, passing through this, makes its way to the outside through the Pyrausta tunnel.

If an artificial opening is made near the anterior end of the cocoon it is not used; the adult chews the usual, and now quite unnecessary, orifice to one side of it through which it emerges.

The adult *Eulimneria crassifemur* is an active, nervous insect which is seldom motionless and is quickly attracted to the light. In the field it can be observed flying rapidly to and fro, pausing on cornflowers to examine with the antennae, or by thrust of the ovipositor, places where Pyrausta larvae are, or have been, feeding. In captivity, especially if confined within a small space, the adults make frantic efforts to escape, beating themselves violently against the sides of the cage. These efforts diminish somewhat if the cage be made larger, and if the insects are placed in a cage high enough to admit a man standing they become somewhat more peaceful and fly around the cage here and there, lighting and crawling upon the walls, mostly toward the light. If sweetened water is given them, the females, and to a certain extent the males, are attracted to it, but when the latter become aware of the presence of a female they cease feeding to engage in mating.

FIGURE 6.—Cocoon of *Eulimneria crassifemur* in tunnel in cornstalk

In captivity both sexes of Eulimneria feed avidly on honey or sweetened water. It is almost certain that they do not as adults feed on the blood or body of the host larva as do so many chalcids, braconids, and some other ichneumonids.

The writers have no data concerning the length of life of this species in nature. Adults which emerged in the Hyères laboratory during the winter from cocoons formed in the preceding fall lived from 9 to 111 days. Table 1 gives the length of life of individuals kept at different temperatures and shows that the average longevity diminishes rapidly as the temperature rises.

TABLE 1.—*Length of life of Eulimneria crassifemur adults at various temperatures, Hyères, France*

Temperature	Length of life of various individuals	Average length of life for fed individuals
°C.	*Days*	*Days*
6.5	ª41, ª47, 65, 106, 111	94
10.0	58, 67	62.5
16.0	18, 41, 42, 59, 70, 77	51.2
23.0	ª33, 41	41
26.0	9, 32	20.5

ª These individuals were not fed. All others were fed at more or less regular intervals.

Copulation may take place immediately after emergence, though in laboratory mating it is found to be a good practice to separate the sexes for from 24 to 48 hours, after which the mutual attraction seems to be more intense. In captivity this species does not mate readily; in small containers, such as glass tubes, lamp chimneys, or small screen-wire cages, the individuals will not mate, but fly about continually and knock themselves against the sides. For successful mating it is essential that the female should remain motionless during a period of preliminary "courtship" by the male. Smearing a few drops of honey or sugar water on the walls of the cage holds the females and allows the preliminary courtship to take place. If, after a period of isolation, the sexes are liberated in a large outdoor cage of screen wire copulation will readily take place. It seems that a moderate breeze blowing upon the parasites facilitates mating. The explanation of this is probably to be found in the fact that the females tend to cling more tightly to the screen in order not to be blown off, and the males are thus able to accomplish their preliminary maneuvers. These observations are substantiated by the behavior of the individuals after liberation in the field. As soon as the "liberating box" is opened the adults begin crawling out on the top and to this they cling for some little time. In a few minutes the top and sides of the liberating cage are covered with mating pairs.

In mating, the male approaches the female and begins fanning her by buzzing his wings rapidly at more or less regular intervals, at the same time raising somewhat the forward part of his body and moving around the female from side to side or in front, gradually approaching her; these movements require from one to two minutes or more for their completion; the male then mounts swiftly upon the back of the female and copulation takes place. Sexual contact continues for about three minutes.

The male is attracted to the female whether or not she has been already fertilized. Males often try, sometimes successfully, to copulate a second time with a fertilized female. A male seems to be able to fertilize several females before his sexual activity begins to diminish.

The exact length of time normally required between emergence and the beginning of oviposition has never been determined. Females have, however, been observed to attempt oviposition within 24 hours after issuing, even though unmated. Dissections of newly emerged females show that there are about 100 fully formed eggs in the oviducts and ovarian chambers.

When fragments of corn plants are placed in a cage with the female she examines them with the antennae, and if Pyrausta larvae have been feeding in the material in question she begins to explore it with the ovipositor, bringing the abdomen forward under the thorax and thrusting the dart here and there among the leaves and flowers. At the beginning of each exploration the sheaths guide the ovipositor to a forward position, whereupon they release their hold and resume their normal position. The exploration is continued until the ovipositor comes in contact with a larva; the dart is then rapidly inserted and an egg deposited. Within from 5 to 10 seconds the dart is withdrawn and the search for caterpillars resumed. The behavior of the female with respect to the hidden larvae of the host strongly suggests that sensorial organs located at the tip of the ovipositor play an important part in the identification of the host.

If a small larva is presented to a Eulimneria female without the protective covering of cornflowers, webs, etc., she will immediately rush upon it, give it a quick thrust with her ovipositor, and be off. The ovipositor is inserted at almost any point of the body, though in the laying cages, owing to the fact that the larvae attempt to crawl away from the direction of the attack, the thrust is often received in the posterior extremity. Sometimes the female deposits only one egg in the host, but often she deposits several, especially if she has been long denied access to hosts.

From 3 to 7 eggs are commonly found in caterpillars collected in the field, as many as 21 having been counted in a single host larva. From one lot of 72 caterpillars parasitized by Eulimneria, 167 eggs of this ichneumonid were obtained by dissection. The concentration of eggs in individual hosts is much too great to be due to chance and can only be attributed to a habit peculiar to this particular parasite.

The sting appears to produce momentary pain, as the host larva squirms and wriggles violently under the thrust, but this soon passes and the victim apparently suffers no permanent ill effect from the sting itself, though if the host is very small—in the first or second stage for example—the sting seems to paralyze, and in some cases the larva does not recover. Even a healthy larva in the third or fourth stage which has been stung repeatedly within a short time will become paralyzed and die.

The Eulimneria female oviposits best in *Pyrausta nubilalis* larvae of stages 3 and 4; she will deposit eggs in stages 2 and 5, though the small larva is sometimes overlooked and the skin of the fifth-stage larva is too thick and tough readily to admit the ovipositor, so the eggs are often found lodged in the skin half inside and half out. In addition, the females seem to have a general reluctance to attempt oviposition in larvae of stage 5.

Larvae of other species seem to attract them but little. In one instance healthy ovipositing females were supplied, in the midst of a series of young *Pyrausta nubilalis* larvae, with small larvae of the lepidopteron *Grapholitha rufillana* Staint., which breeds in the heads of wild carrots (*Daucus carota*). The females rushed at the Grapholitha larvae as they had been in the habit of doing with the *Pyrausta nubilalis* larvae, but just at the moment of oviposition they seemed to perceive something wrong and passed rapidly on to another object, some individuals giving a half-hearted thrust, others refraining

completely. A few eggs were deposited in these larvae but none of the resulting parasites came through to maturity.

HATCHING AND GROWTH OF LARVA

In a very interesting paper published in 1926, Cushman (*3, pp. 42–43*) deals with the various types of parasitism among the Ichneumonidae with particular reference to larval form and habits. This species on account of its habits and larval anatomy falls into Cushman's third type of internal parasites, along with *Limnerium validum* Cress., *Campoplex pilosulus*, and other campoplegines.

The eggs float freely in the body cavity of the host, but by reason of the peristaltic movements of the intestine and the circulation of the blood they soon come to be lodged in the extreme posterior end of the larva among the Malpighian tubes.

The eggshell splits open at one end and allows the young larva to squirm its way out. The shell remains with other fragments of débris packed in the posterior part of the host's body cavity and can be seen even when the Eulimneria larva has obtained its full growth. It is usually surrounded by a mass of phagocytes.

When several eggs are laid in one host larva only one Eulimneria survives to maturity. Their remains indicate that the surplus larvae are killed off by the first Eulimneria to hatch, or at all events by one of the earlier ones; it is known positively that the individuals which succumb all die very soon after hatching as they are often found dead only partly emerged from the egg and always in the first stage. In one instance a partially emerged larva dead with the mandibles of another first-stage larva fixed firmly in its body was observed. In this case the attacking larva also was dead, as well as a number of other first-stage larvae of Eulimneria in this same host.

It seems unlikely, however, that the death of the supernumerary individuals is in general due to a series of combats between the larvae. It is more probable, as Spencer (*11, p. 134*) has suggested in the similar case of Aphidius, that at a certain moment, soon after the hatching, the larvae begin to pour into the blood a cytolytic enzyme which affects the tissues of the host, and those of the younger larvae of Eulimneria itself.

The presence of a live Eulimneria larva in the body cavity of the host caterpillar does not seem to affect the health of the latter, at least during the parasite's first stage, for during this period the parasite is very small and seems to procure its nourishment without attacking the organs of its host. As the parasite larva grows it molts at least twice, and by the time it reaches the third stage it occupies three-fourths of the body cavity of its host. At about this stage the Eulimneria larva absorbs the material of the host's organs and when full fed it pierces a small hole in the skin of the now dead corn borer, pushes its way out, and spins its cocoon, to which the old host skin usually clings by a few threads. If the parasite larva be removed from the Pyrausta tunnel and placed upon a flat surface it is unable to spin a cocoon but scatters its silk over the surface. This is also true of a great many of the spinning larvae.

The cocoon may be opened toward the end of the pupal period without necessarily causing any ill effects to the larva, but if the larva is removed shortly after it has finished spinning it usually dries up and dies as do larvae which have been unable to form their cocoons.

When the winter is past, and the rise in temperature determines the resumption of activity, the larva expels its meconium, sheds its skin, and transforms into the typical white hymenopterous pupa. This pupa gradually darkens until the completely formed adult breaks out of the pupal skin and issues as already described.

HIBERNATION

In a paper published several years ago on the phenomena of hibernation in the Muscidae, E. Roubaud (*6, p. 469*) classified insects into two categories, the homodynamic, which included species capable under favorable conditions of uninterrupted development for an indefinite number of generations, and the heterodynamic, which included species in which an obligatory resting period occurs in some stage or other at periodical intervals in the succession of generations. Hibernation or aestivation are in the homodynamous species determined purely by external conditions such as low temperature or dryness, while in the heterodynamic forms they are determined essentially by the physiological condition of the organism.

Eulimneria crassifemur is to some extent intermediate between these two types. The prepupal and pupal period of the summer generation is completed in from about 15 to 20 days. In the cocoons of the winter generation this phase may require four or five months. The recurrence of the diapause is not, however, independent of environmental conditions, for in the 1-generation areas it occurs in every generation, while in the 2-generation areas it occurs only once every two generations and only in the larvae which spin cocoons in the fall. Furthermore, unlike certain species of the heterodynamic group described by Roubaud, *E. crassifemur* is susceptible, even during the diapause, to the influence of high temperature. Thus a lot of cocoons collected in southwestern France on January 1, 1927, and kept in cold storage until February 10 were on that date divided into two lots, one of which was kept at 18° C. and the other at 27° C. The results of this experiment were that adults emerged from those kept at 27° C. in 11 days, whereas those kept at 18° C. did not produce adults until the twenty-second day. The exposure to high temperature thus produced a well-marked acceleration of development.

As the season advances, the time required for the completion of the pupal stage at a given temperature gradually decreases, proving that under field conditions the hibernating individuals are slowly developing. This is shown clearly in Table 2, which gives the results obtained by the incubation at 33.5° C. of cocoons collected at Auch, France, on a series of dates from December 29, 1924, to February 2, 1925. It will be noted that the number of days from the beginning of the experiment (January 5) to emergence increases as the date of collection advances, the reason for this being that development went on less rapidly in the specimens which remained in the field. The specimens placed at 33.5° C. on January 5 completed their development in only 10 days less than those taken from the field on February 2.

TABLE 2.—*Accelerated emergence of adults of Eulimneria crassifemur from cocoons placed at a temperature of 33.5° C. at different periods of natural development at Hyères, France*

Date of collection	Date placed in incubator [a]	Number of days from placing in incubator to emergence	Day of emergence counted from beginning of experiment on Jan. 5
Dec. 29, 1924	Jan. 5, 1925	33	33
Jan. 1, 1925	Jan. 7, 1925	31	33
Jan. 6, 1925	Jan. 13, 1925	26	34
Jan. 12, 1925	Jan. 19, 1925	14	28
Jan. 20, 1925	Jan. 27, 1925	13	35
Jan. 29, 1925	Feb. 5, 1925	9	40
Feb. 2, 1925	Feb. 9, 1925	8	43

[a] During the interval between the date of collection at Auch and the beginning of the incubation at Hyères the cocoons were in transit and at a temperature probably slightly higher than in the field.

SEASONAL HISTORY

Eulimneria crassifemur occurs over the range of *Pyrausta nubilalis* in the 1-generation and "transitional" zones and in certain of the 2-generation zones of the latter. Throughout these zones the seasonal history of Eulimneria coincides more or less with the seasonal history of *P. nubilalis* except for the fact that in the transitional zones the parasite usually has only one generation with no partial second, whereas *P. nubilalis* may have a partial second even in unfavorable seasons. If the season is favorable, causing the host to pass through two full generations, the Eulimneria may have a partial or entire second generation. Such a partial second generation occurred at Auch, France, in 1920.

The seasonal history of Eulimneria for the various zones in which it has been studied (fig. 7) may be summarized as follows:

AQUITANIAN ZONE (SOUTHWESTERN FRANCE).—In this zone there is usually one generation of *Pyrausta nubilalis* and one of Eulimneria. The majority of the adults of Eulimneria emerge between March 15 and April 15, the heaviest emergence being about April 1. Some few individuals emerge earlier or later,[6] the earliest record being February 9 and the latest May 26 (though one white nymph was found in its cocoon on June 6).

The writers believe that these adults wait about until the young *Pyrausta nubilalis* appear, about the 1st of August, and oviposit in them. (A spring generation in some intermediate host is possible, though, in the writers' opinion, unlikely.) The resulting parasite larvae reach maturity by the end of September or the middle of October, spin their cocoons, and remain over winter in the dried cornstalks as fully developed larvae, transforming into nymphs and adults the following spring.

If the season be very early and warm, the spring emergence of the host is somewhat advanced, as is also that of the parasite to a certain extent. This permits an entire or partial 2-generation cycle. In this case the earliest larvae of *Pyrausta nubilalis* emerge during the first half of July and are then parasitized by Eulimneria. The resulting

[6] This seems to be an important point in the life history of Eulimneria. The writers believe that only the late-emerging individuals can live long enough to encounter the earliest host larvae. This matter will be discussed more fully later in this paper (p. 339).

generation of the parasite completes its development and emerges during the latter half of August, at which time, or shortly thereafter, young larvae of *nubilalis* of the second generation are present in the tips of the ears, at the bases of the greener leaves, and in fresher parts of the corn plant. The parasites oviposit immediately in these young caterpillars, and the cocoons of this generation are all spun by the 1st of November. The winter is then passed in the cocoon as in the case of the 1-generation cycle.

MEDITERRANEAN (NORTH) ZONE (RHONE VALLEY AROUND VALENCE.)—In this region there is regularly only one generation of *Pyrausta nubilalis* with sometimes a small partial second generation amounting to 10 or 15 per cent of the total. With Eulimneria, however, there is only one generation so far as is known. The Eulimneria

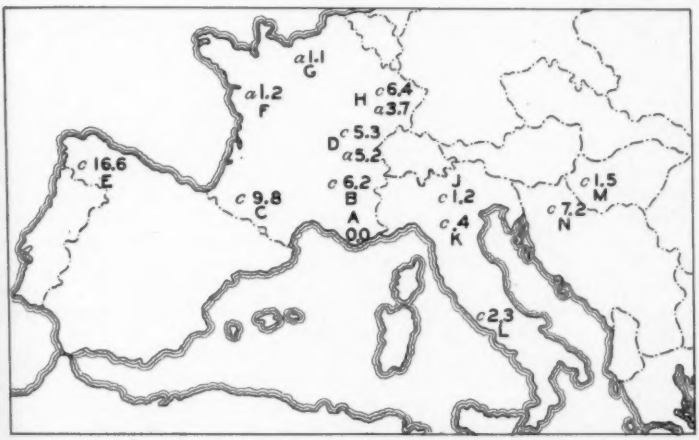

FIGURE 7.—Map showing the zones in Europe in which *Eulimneria crassifemur* has been studied: A, Mediterranean (south); B, Mediterranean (north); C, Aquitanian; D, Rhodanian; E, Galician; F, Armorican; G, Sequanian; H, Vosgian; J, Padovian (north); K, Padovian (south); L, Campanian; M, Hungarian; N, Yugoslavian. The figures represent the average percentage of parasitism of *Pyrausta nubilalis*. *a*, in Artemisia; *c*, in corn

cocoons from the first-generation hosts are formed in August, but no adults issue from them until the following spring.

SEQUANIAN ZONE (PARIS, LILLE).—In this zone there is only one generation of host and parasite. The adults issue at about the same time as, or but slightly later than, they do in the Auch (Aquitanian) region, and the eggs are laid in late July and early August, at which time larvae of *nubilalis* are available. The development of these larvae proceeds more slowly (as does that of the host) than in either the Aquitanian zone or in that of the Rhone Valley, the cocoons being formed during the latter part of September and early October. The plant in which *P. nubilalis* develops in this zone is *Artemisia vulgaris*.

ARMORICAN ZONE.—In the Armorican zone the seasonal history is practically identical with that of the Sequanian.

VOSGIAN ZONE (COLMAR, STRASBOURG).—In the Vosgian zone, so far as known, the seasonal history of Eulimneria is the same as at Paris (Sequanian zone). Both the host and parasite may occur,

however, in corn as well as in Artemisia. Young Eulimneria of the first and second stages have been found near Strasbourg at the end of August and in early September.

RHODANIAN ZONE (JURA).—In this zone there is but one generation of host and parasite. The seasonal history here differs from that of the Rhône Valley zone (Valence) by the fact that oviposition is somewhat later, development slower, and the cocoons formed toward the end of September and during October.

DANUBIAN ZONE (HUNGARY, YUGOSLAVIA).—This is also a 1-generation zone, resembling the Sequanian, the Rhodanian, and the Vosgian zones in that the larvae of both host and parasite develop very slowly. Young host larvae are present from the middle of July on to September, while the Eulimneria eggs are laid during the latter part of July and early August, the earlier stages (eggs and first-stage larvae) being found in the host as early as August 10 or 12 and as late as September. The cocoons are spun during the latter part of October and the early part of November. In years in which the season is advanced the development may be somewhat earlier, as in 1927 eggs and young larvae of Eulimneria were found by A. M. Vance early in July.

PADOVIAN ZONE (PO VALLEY, BERGAMO, PIACENZA, PAVIA, ETC.).—In this zone *Eulimneria crassifemur* has two distinct generations, corresponding more or less with the two host generations. Eggs of the first generation are laid in early July while the cocoons, which may be of either the thick or thin type (see p. 330), are formed during the latter part of July and the early part of August. The adults issue from August 15 to 31 and deposit eggs of the second generation. The overwintering cocoons are formed during the latter part of October and early November and the adults issue the following spring during May and even until as late as June 5.

CAMPANIAN ZONE (NAPLES).—The data for this region are somewhat meager, but it is known that there are at least two generations. Adults issue in the spring from February to April and perhaps later. As Eulimneria does not exist at Hyères on the French Riviera, the Mediterranean (south) zone, where the climate closely resembles that of Naples, the writers are led to believe that at Naples the Eulimneria adults migrate down from the inland valleys where a milder summer prevails. Cocoons of the first generation have been collected late in July and early in August.

The seasonal history of *Eulimneria crassifemur* is thus approximately as follows: In the typical 1-generation zones (Sequanian, Armorican, Vosgian, Rhodanian, and Danubian) the adults issue in the spring from late February until late May, with the maximum emergence about April 15, and lay their eggs in the earliest host larvae during a period which may commence in early July and extend on until as late in the season as early September. During this time the progeny develop slowly, and the cocoons are formed in October and early November.

In the 2-generation zones (Padovian and Campanian) the adults issue in the spring at about the same time as in the 1-generation zones, laying, in late June and early July, eggs which develop more rapidly, producing adults in August which oviposit for the second generation. The cocoons of these are formed toward the end of October and pass through the winter.

In the transformation zones (Aquitanian and Rhône Valley) the oviposition may occur at the same time as in the single-generation

zones, and development may proceed, according to the temperature, (1) slowly or (2) rapidly. If it proceeds slowly there will be but one generation, the cocoons being formed in late September, October, and early November; if it proceeds rapidly cocoons may be formed and adults issue about the middle of August, in which case there will be two generations. If the development is more rapid than in (1) and less rapid than in (2), cocoons may be formed in August, and instead of giving adults at once they may go over the winter, furnishing adults the following spring. This latter condition has been encountered in the Rhône Valley (Mediterranean, north) zone but never in the Aquitanian.

GEOGRAPHICAL DISTRIBUTION IN RELATION TO CLIMATE

The study of *Eulimneria crassifemur* in Europe has led to the conclusion that the presence or absence of this species in a given region depends more upon temperature than upon any other meteorological factor.

As has already been shown (Table 2), the hibernating individuals, though they are more refractory to external influences than those of the summer generation, are nevertheless affected by a rise in temperature, which produces a definite acceleration of development.

The hibernating larvae of *Pyrausta nubilalis* are, however, much less responsive to variations in external conditions. Exposure to high temperature during the diapause (during the months of January and February, for example) produces simply the desiccation and death of the majority of the larvae and determines in those which survive a relatively slight acceleration of development. After the larvae have emerged from the diapause, variations in temperature have, of course, the same effect as upon any homodynamous insect.

The result of this difference between Eulimneria and Pyrausta is that rises in temperature during the hibernation period, i. e., warm winters, determine an asynchronism in the seasonal histories of the parasite and its host. In a warm winter the parasites will thus emerge and die long before any larvae of the host are available.

In regions where the accumulated temperatures during the hibernation period are sufficient to produce emergence early in the winter *Eulimneria crassifemur* is likely to be uncommon or absent unless alternate hosts can be utilized.

In the southern Mediterranean zone the winters are mild and warm days sufficiently frequent to cause a very early emergence (late October or early November) of Eulimneria. This climatic feature, coupled with the fact that few lepidopterous larvae that would be suitable as hosts exist in the field at this time, is taken to be the reason for the complete absence of the parasite in this zone.

The occasional appearance of *Eulimneria crassifemur* in Naples, where the climate is essentially similar to that of the southern Mediterranean zone, may be explained by an annual migration of the Eulimneria from the higher valleys behind Naples down toward the lowlands. At all events these valleys with their more severe winter would serve as a constant source of supply for the Campanian zone, whereas the southern Mediterranean zone is entirely cut off from any such districts by mountains, waste lands, and vineyards from which *Pyrausta nubilalis* is practically absent.

This hypothesis does not, of course, explain the variations in the apparent effectiveness (i. e., relative abundance and percentage of parasitism) of Eulimneria in the other zones where it is found. Many other factors would have to be considered before the causes of these variations would become clear. The reaction to temperature of the hibernating individuals in regions where the species has a 1-generation seasonal history may be quantitatively different from that of the hibernating individuals of the 2-generation zones. The attraction of the adult parasite to larvae in weeds such as *Artemisia vulgaris* may be less intense than toward larvae feeding in corn. In certain regions the species may have other hosts to which a considerable proportion of the population is attracted in every generation. The question of the abundance of this species in the various parts of its habitat is thus one of extreme complexity and probably has no simple solution applicable to all conditions.

EULIMNERIA CRASSIFEMUR AS A FACTOR IN THE CONTROL OF PYRAUSTA NUBILALIS IN EUROPE

Eulimneria crassifemur is one of the most widely distributed of the parasites of *Pyrausta nubilalis* in Europe and is constantly present in all of the zones except the Mediterranean (south). Table 3 shows its relative importance as a whole in the various zones and should be consulted in connection with the map (fig. 7).

TABLE 3.—*Average and maximum parasitism by Eulimneria crassifemur in Europe during the period of investigations (the maximum parasitism was always by the first generation)*

Zone	Average parasitism	Maximum parasitism	Host plant	Year in which maximum parasitism occurred
	Per cent	*Per cent*		
Galician [a]	16.60	16.60	Corn	1925
Aquitanian	9.87	27.90	do	1920
Yugoslavian	7.20	13.20	do	1926
Vosgian [a]	6.40	6.40	do	1925
Mediterranean (north)	6.20	6.20	do	1925
Rhodanian	5.35	5.70	do	1924
Do	5.20	9.00	Artemisia	1925
Vosgian [a]	3.70	3.70	do	1925
Campanian	2.30	2.60	Corn	1924
Hungarian	1.58	3.30	do	1926
Padovian (north)	1.24	3.00	do	1924
Armorican [a]	1.20	1.20	Artemisia	1925
Sequanian	1.10	3.50	do	1925
Padovian (south)	.40	.40	Corn	1925
Mediterranean (south)	00	00		

[a] Only 1 year's observation available.

Table 4 shows in detail the percentage of parasitism of Pyrausta by *Eulimneria crassifemur*, by year and by generation, observed in corn and in Artemisia in the various zones during the years in which it has been studied. It should be pointed out that while the figure for the average parasitism in the Galician zone is higher than in any other region this figure is based upon a single year's observation and is likely to be misleading for the reason that *Pyrausta nubilalis* is extremely scarce in this zone. In reality Eulimneria is much more abundant in the Aquitanian zone than in any other region studied.

TABLE 4.—*The percentage of parasitism of Pyrausta by Eulimneria crassifemur, by years and generations, as observed in corn and Artemisia in the various zones of Europe, 1919–1927*

Year and generation	Mediterranean (north)	Aquitanian	Rhodanian		Galician	Armorican	Sequanian	Vosgian		Padovian (north)	Padovian (south)	Campanian	Hungarian	Yugoslavian
	Corn	Corn	Corn	Artemisia	Corn	Artemisia	Artemisia	Corn	Artemisia	Corn	Corn	Corn	Corn	Corn
1919:														
Generation 1														
Generation 2		6.90												
1920:														
Generation 1		27.90												
Generation 2		9.90												
1921:														
Generation 1														
Generation 2		6.00												
1922:														
Generation 1							1.19							
Generation 2		3.50												
1923:														
Generation 1							1.00							
Generation 2														
1924:														
Generation 1		4.30	5.70	1.07			1.00			3.00		2.60		1.50
Generation 2										.90				
1925:														
Generation 1	6.20	2.90	5.00	9.00	16.60	1.20	3.50	6.40	3.70	2.50	0.40	2.50	1.00	1.50
Generation 2														
1926:														
Generation 1		3.37				.70	.34			.50			3.30	13.20
Generation 2														
1927:														
Generation 1		2.40				.43	.84			3.12				
Generation 2										.01				

LIMITING FACTORS IN THE EFFECTIVENESS OF THE PARASITE

A parasite having an effective reproductive rate which is equal to or greater than that of its host, and which increases at the expense of the latter from generation to generation, must eventually come to equal it in numbers and almost exterminate it.

A parasite having an effective reproductive rate equal to that of the host, and whose population at the beginning of the period considered is numerically equal to one-fourth of the host population, should bring about the practical extermination of the host in four generations (Thompson, *14*, *15*, *16*).

In the first generation of *Pyrausta nubilalis* in 1920 in southwestern France the population of Eulimneria larvae was, according to estimates based on 20,000 corn-borer larvae actually examined, more than one-fourth that of the host. If the Eulimneria reproduces at the same rate as the corn borer, it should, in the third generation following, have increased to the point where it would parasitize practically 100 per cent of the host.

What actually occurred was that the proportion of caterpillars parasitized by Eulimneria fell in the succeeding generations and has not, as far as known, been above 10 per cent in the seven years following 1920. During this time no marked increase in the population of Pyrausta has occurred. The writers are therefore obliged to conclude that Eulimneria does not reproduce even as rapidly as Pyrausta, but in fact much less rapidly.

Furthermore, as the data in Table 4 show, in no region where this insect has been studied has there been any real indication that Eulimneria is increasing at the expense of Pyrausta.

As the parasitic habit constitutes a distinct advantage for Eulimneria (since every Eulimneria emerging means at least one less Pyrausta), it follows that the survival of Eulimneria as a parasite of Pyrausta must be very seriously inhibited by limiting factors.

An important limiting factor is probably the low reproductive rate of *Eulimneria crassifemur* as compared to Pyrausta. The writers have as yet no accurate data as to the number of eggs ordinarily deposited by *E. crassifemur*, but dissections of females lead them to believe that a potential total of 500 eggs is present. It is certain, however, that, as is usual among insects, a large proportion of these eggs are not deposited.

Paillot (*5*, *p. 191*) estimates that the form studied by him contained in its ovaries not less than 300 eggs but states that the number of eggs actually deposited never attains this total, for the female succumbs before her ovaries are exhausted. The average number of eggs actually deposited by *Pyrausta nubilalis* seems, however, to be well over 300 according to Caffrey and Worthley (*2*, *p. 108*).

The wasteful distribution of eggs by *Eulimneria crassifemur* is also unfavorable to the species. The results so far obtained indicate that probably one-half the eggs deposited by the females are placed in caterpillars already containing at least one egg. As has been seen, only a single Eulimneria larva survives in such cases and in many instances all die.

The Eulimneria female possesses a moderately long ovipositor and is able with the aid of this organ to discover larvae of the corn borer concealed within the various parts of the corn plant. Nevertheless

the position of the caterpillars must make them in many cases quite inaccessible to the parasite. The proportion of larvae thus escaping attack is usually greater in the second generation than in the first, for in the first generation the larvae or their castings are more or less apparent in the young flowers, whereas in the second generation they are hidden away in the stalk or lie deep between the stalk and leaf base or have burrowed far into the midrib of the leaf, or into the ears where it is impossible for the Eulimneria adults to find them. As a matter of fact, as the data tabulated show, the parasitism by this species in the caterpillars of the second generation is normally much lower than in the first generation.

Secondary parasites, among which are *Pezomachus* spp., *Dibrachys* sp., and *Melittobia acasta* Walk., have been reared from Eulimneria cocoons, but these constitute a negligible limiting factor.

Finally, the susceptibility to high temperatures during the winter months, causing in many years the premature emergence of the adult parasites, must, in many regions, greatly reduce the effectiveness of the species.

A compensating factor is the late burning of cornstalks, a practice common in the southwestern corn region of France. If the stalks are burned early with both the parasite and the host still in them, both suffer the same relative diminution, but if they are burned after the parasite emerges and before the corn borer emerges, the relative abundance of the parasite would be decidedly augmented.

Other limiting factors, still unknown to entomologists, probably operate with greater or less intensity in certain seasons and are responsible for certain fluctuations that have been observed. There can, however, be little doubt that those already mentioned suffice to limit very effectively the work of *Eulimneria crassifemur* as a parasite of *Pyrausta nubilalis*, so that, though the parasite certainly plays a definite part in the control of Pyrausta over the greater part of its range, it is incapable alone of reducing an outbreak or of producing a notable decrease in the damage resulting from the work of this pest.

SUMMARY

Eulimneria crassifemur Thomson (Hymenoptera, Ichneumonidae) is an important parasite of the European corn borer in Europe, where distribution records have been obtained from Spain, France, Belgium, Germany, Sweden, Switzerland, Italy, and the central European plains.

The adult is a black-bodied, red-legged, wasplike insect about one-half inch long. The egg is simple, oblong-ovate, white when first deposited but turning black after several days. The first-stage larva is slender, segmented, possessing a rather heavily chitinized brown head with strong hooked mandibles and a long pointed caudal segment; it has well-developed internal organs, tracheal trunks and branches but no open spiracles. The tail appendage is not a tracheal gill, as some have supposed. The mature larva is dirty white, of the typical "hymenopteriform" type with well-differentiated mouth parts, simple mandibles, and nine pairs of open spiracles. The winter cocoon is thick and gray or black, and the summer cocoons are often thin and white with a light band around the middle.

The adults will feed on honey or sweetened water, but not on the body or blood of the host larvae. They may live as long as 111 days at a low temperature. The newly-emerged female will have about 100 fully formed eggs in the ovaries and ducts, and can deposit altogether as many as 300 eggs.

The egg is deposited in the body cavity of the young host larva, and the parasite larva lives free in the host, destroying it by absorbing the internal organs as the larva approaches maturity. The parasite emerges from the old borer skin and spins its cocoon in the host tunnel. Development proceeds slowly during hibernation.

Generally there is but one generation a year; the adults from the overwintering cocoons emerge from March to June and lay their eggs in the earliest appearing corn-borer larvae in July or August. Only the latest emerging adults live long enough to find Pyrausta larvae in which to oviposit, for by the time these appear the majority of Eulimneria adults have probably died. So far as the writers know there is no intermediate host. The larval growth takes place in summer, and the overwintering cocoons are spun in September or October.

Eulimneria crassifemur clings feebly to a seasonal rhythm, but this can be broken by a considerable fluctuation in the temperature factor. Thus temperature seems to be the most important single climatic factor influencing its range. The parasite does not occur on the French Riviera, as the prevailing high temperature probably prevents a successful seasonal rhythm. The fact that it occurs around Naples may be accounted for on the theory that it migrates annually, or at least at intervals, from the higher mountain valleys.

Eulimneria crassifemur is found in the corn borers in both corn and Artemisia. The maximum parasitism of the host that has been recorded in Europe is 27 per cent.

Limiting factors that may hinder the abundance of this species are a lack of perfect synchronization between host generations and parasite generations, superparasitism, or the laying of more than one egg in the same host, and cold, wet weather during the oviposition period. A few secondary parasites have been reared, but they are negligible as far as influencing the numbers of the parasite is concerned.

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THE POSSIBLE TOXICITY OF GRAIN-SORGHUM SMUTS[1]

By V. G. HELLER and CHARLES CASKEY, *Department of Agricultural Chemistry Research*, and ROBERT PENQUITE, *Poultry Department, Oklahoma Agricultural and Mechanical College, Stillwater, Okla.*[2]

INTRODUCTION

The grain sorghums have proven to be especially adapted to some of the more arid sections of this country where they are grown and fed in large quantities to livestock. Unfortunately many members of the group are subject to kernel smut infections. These infections can be partially avoided by proper treatment of the seed with copper carbonate or other fungicides, but the treatment is often omitted, with the result that in some years as high as 70 per cent of the heads in certain fields are affected. In the same sections of the country the small grains are often attacked by the fungus ergot, which is distinctly toxic to livestock.

When the dried heads are handled or threshed, the smut spores are liberated in the form of a very fine dust. The writers have noted peculiar physiological reactions upon its inhalation. The upper pulmonary tract was sensitive to the passage of air, the heart action seemed to be increased, these symptoms being followed a few hours afterwards by headache and a partially nauseated feeling. For these and other reasons, it has been thought that deleterious results might be associated with the consumption of smut spores. Livestock feeders have often hesitated to use feed thus affected, and when death did occur among the cattle, it has been attributed to this cause.

An investigation of the literature has failed to reveal any definite information in regard to the effect of grain-sorghum smut on livestock. The only related work consists of studies of corn smuts. Pammel [3] states that corn smut is supposed to be poisonous to cattle, in some forms ergotin is found, while the Bureau of Animal Industry [4] finds that smut is not toxic to heifers. This finding is in accord with that of Smith [5], who fed smutty corn to cows without injury, in fact the cows seemed to relish it. Henry [6] fed large quantities to cows for a considerable time. They fattened and did well until one unaccountably died. Hutyra and Marek [7] state that smut produces gout in chickens. However, the smut of corn is of an entirely different variety, and the sorghum smut might have properties in common with the toxic smuts of small grains.

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[2] The writers wish to express their appreciation to H. H. Finnell, in charge of the experiment station at Goodwell, for his assistance in gathering and classifying the smut.

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For these reasons it has seemed advisable to conduct a series of experiments in order to obtain some definite information in regard to the effect of grain-sorghum smut on the health of animals when it is present in their feed.

EXPERIMENTAL METHODS AND PROCEDURE

During the year 1927, some sorghum fields were visited in which as high as 70 per cent of the heads were smut-infected to a greater or less degree. Before harvesting the crop, heads in which practically all the kernels were filled with spores were gathered and dried. These heads were threshed by hand and the smut spores separated by the use of fine screens. This smut weighed 34.1 per cent as much as the grain in an equal number of noninfected heads. Partial analysis of the air-dried material gave the following results:

	Per cent
Water	9.00
Ash	8.42
Proteins	13.5
Dextrose	1.03
Invert sugar	.07
Lost with ether extract	.76
Lost with 95 per cent alcohol following ether extract	.66
Water soluble following ether and alcohol extract	20.26

FEEDING EXPERIMENTS WITH RATS

In order to determine whether or not the smut spores would be injurious when fed to animals, experiments were planned whereby a comparison could be made of the growth and reproduction of various kinds of animals receiving a complete well-balanced basal ration and a second series receiving the same rations supplemented with various amounts of the smut spores. In the first tests rats were used as experimental animals. Healthy young rats about 4 weeks of age were chosen and placed in the cages regularly used for nutrition tests, care being exercised to place a lot comparable in litter origin, sex, and size in each cage. A ration was planned so as to be similar to the one consumed by animals eating the whole grain and yet be complete in proteins, vitamins, and minerals.

The percentage constitution of the basal ration was as follows: Kafir, 80; tankage, 8; ground alfalfa, 5; NaCl, 1; $CaCO_3$, 1; and cod-liver oil, 5.

One lot of rats used as a control series were fed this ration throughout the experiment and records were made of their growth, age of reproduction, the number of young, and the development of offspring. Similar lots received the same ration in which varying quantities of smut spores replaced like quantities of carbohydrates in such manner as would cover the usual range of contaminated heads.

The general results are to be found in the growth charts. Figure 1 represents the growth of animals receiving the basal ration. Growth and reproduction were normal in every case.

Figure 2 represents the growth and reproduction of animals receiving the basal ration supplemented with 8 per cent smut. It will be observed that the growth was fully as satisfactory as in the case of those receiving the basal ration alone. It will be further observed that the females became pregnant and gave birth to young. The

ergot of small grains seems to cause an abnormality in pregnant mothers, abortion being common. The number of young rats in the litter was as great as is usually found in normal animals. There were some losses by death among the lots receiving the basal ration and slightly higher death rates prevailed among those receiving the smut. An examination, however, failed to reveal any symptoms of toxic conditions. It was concluded that the death of some young might be due to the fact that the fiber content of the feed was higher than is desirable for rat feeding.

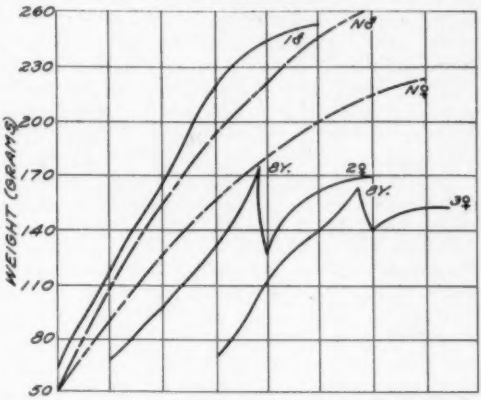

FIGURE 1.—Growth and reproduction of rats on a complete basal ration. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N and Y signify normal and young, respectively. Spaces between ordinates represent 4-week periods

Figure 3 records similar results for smut feeding in the second generation. Records of third and fourth generation animals, though not charted in the

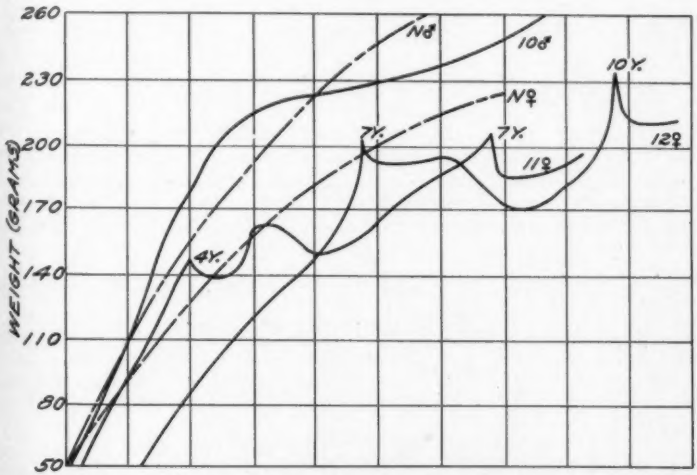

FIGURE 2.—Growth and reproduction of rats on a complete basal ration to which 8 per cent of sorghum-smut spores had been added, replacing carbohydrates in like amount. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N=normal, Y=young. Spaces between ordinates represent 2-week periods

figures, have been likewise made, and there was no evidence of deleterious effects from feeding smut spores.

EXPERIMENTS WITH OTHER SMALL ANIMALS

It was then decided that even though the smutty grain might not be injurious to rats it might be toxic to other animals, so experiments were planned using both rabbits and guinea pigs. In this case the percentage composition of the basic ration was as follows: Ground kafir, 59; wheat, 30; alfalfa, 5; milk powder, 5; NaCl, 1; and $CaCO_3$, 1. Green lettuce or cabbage was added twice a week. Six per cent smut was substituted for an equal amount of kafir in the smut test rations. Both the guinea pigs and the rabbits ate the rations readily. No apparent difference could be observed between the animals consuming the basal ration and the smutty food. Growth and reproduction were normal. The average increase in weight per week was 18 gm. for guinea pigs and 134 gm. for the rabbits, this increase being

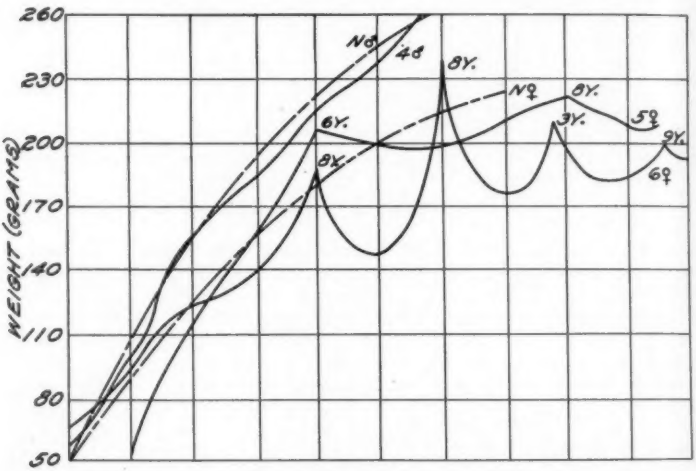

FIGURE 3.—Growth and reproduction of second-generation rats which received the complete basal ration to which 10 per cent of sorghum-smut spores had been added, replacing carbohydrates in like amount. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N=normal, Y=young. Spaces between ordinates represent 2-week periods.

practically uniform throughout the test period. One of the rabbits gave birth to nine young.

Inasmuch as kafir furnishes an ideal feed for chickens, an extended study is being made with laying hens and growing chickens. Even though the smut was present in such large quantities that the chickens' throats became black with it, no deleterious effects have so far been noted.

FEEDING EXPERMENTS WITH HORSES AND COWS

Having demonstrated under carefully controlled conditions that the smut spores seemed to have no ill effect on small experimental animals, it was next desirable to observe the effects on farm animals as fed under farm conditions, or when the rations were somewhat inadequate, as is often the case under these conditions. A farmer was found who had broadcast his sorghum seed. The sorghum plants were somewhat thick, the heads were small, and fully 70 per cent of

them were smut-infected. The entire plant was cut and bound, the seed and smut remaining in the head, and fed to horses, milk cows, and young cattle.

The horses were mature animals used for farm work and received only the sorghum fodder including the smutty heads, together with yellow ear corn, salt, and water. After 12 weeks of such a limited diet, no ill effect could be observed. During a greater portion of the time the horses were used for heavy hauling.

The milk cows were fed a pound of soybean meal a day, as much of the sorghum fodder as they desired, together with salt and water. Nine young cattle received only fodder, containing the smutty grain in head, salt and water. Six of these gave birth to normal calves at the normal time. All of the animals maintained themselves as well as could be expected on so limited a ration. It is thought that the tests demonstrate beyond doubt that the smut possesses no toxic principles when fed in as concentrated a form as ordinarily found in the field, and expecially was this the case since the animals were fed a ration the efficiency of which was limited by the character and quantity of its proteins and vitamins. It is held to be quite generally true that when animals are receiving a somewhat inadequate ration they become more susceptible both to diseases and toxic substances than when better nourished.

SUMMARY AND CONCLUSIONS

Certain types of grain sorghums often have as high as 70 per cent of the heads affected with kernel smuts which replace the grain kernels.

Biological tests using rats, guinea pigs, and rabbits failed to reveal any deleterious results in growth, reproduction, and rearing of young when as high as 10 per cent of smut spores replaced an equal amount of carbohydrates in an otherwise adequate diet.

Chickens were fed satisfactorily on rations prepared from smutty seed.

Horses, cows, and young cattle were fed smutty sorghum grain and fodder without displaying any symptoms of toxicity.

During the observation of all these animals (over 65 in number) no sickness or deaths occurred. Young animals grew as well as the controls. Old animals maintained their weight. Reproduction took place at normal periods with four types of animals and neither the egg production of hens nor the milk production of cows was altered by feeding sorghum smut in a form as concentrated as it occurs in the field. This was true with larger farm animals even when their ration was somewhat inadequate.

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN WHITE ODESSA WHEAT[1]

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INTRODUCTION

The inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., in Martin and Hussar wheats has been described in previous publications (*2*, *3*).[2] Since the literature pertinent to the inheritance of resistance to bunt has been reviewed and discussed in these publications, it will not be repeated here.

Martin and Hussar were the only two varieties of wheat found to be completely resistant to bunt in an extensive varietal test conducted in the Pacific Coast States (*10*). However, a few other varieties were highly resistant, usually producing less than 10 per cent of diseased heads. White Odessa (C. I.[3] 4655) was such a variety, with an average of only 2 per cent of bunted heads at the Washington, Oregon, and California Agricultural Experiment Stations, and an average of 2.3 per cent of bunted heads at the California station. However, the selection of White Odessa used in this investigation has been completely resistant to bunt at Davis, Calif., in the 3-year period beginning in 1926.

In 1925 White Odessa was crossed with White Federation, which is very susceptible to bunt. The data obtained in a study of the F_2 and F_3 progenies are described in this paper.

The inoculum was from the same collection of bunt as that used in 1920 and 1921. The original collection may have consisted of two or more physiologic forms, one of which could attack White Odessa slightly. It is conceivable that this form may have been lost in propagating the inoculum on White Federation wheat. In any case, the genetic difference between a completely resistant selection of White Odessa and one which produces 2 per cent of bunted heads probably is not very great.

White Federation may be considered completely susceptible as compared with White Odessa. Three rows contained an average of 80.8 per cent of bunted plants in 1926, 13 rows an average of 66.6 per cent in 1927, and 12 rows an average of 68.9 per cent in 1928. The average for the 9-year period beginning in 1920 was 71.4 per cent of bunted plants.

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there were especially suitable for these investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both

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[2] Reference is made by number (italic) to "Literature cited," p. 359.

[3] C. I. indicates a serial number of the Office of Cereal Crops and Diseases.

spring and winter varieties may be seeded at that time of year without any danger of winterkilling and with the assurance that both types will mature in the following summer.

In the present investigation the seeds were thoroughly blackened with bunt by placing an excess quantity of the spores with the wheat in a glass container and shaking vigorously. The inoculum was originally collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hills district of Solano County, Calif.

PARENTS AND METHODS

White Odessa wheat (C. I. 4655) produced 2 per cent of bunted heads at Davis in 1920 and 2.7 per cent in 1921. From 1921 to 1925 this variety was propagated each year from seeds from single plants in the parent wheat nursery and was not subjected to bunt infection. In 1925 the flowers of one head were crossed with pollen from White Federation wheat. The other heads on that plant have been the source of seed of White Odessa wheat used since that time.

During the 3-year period beginning in 1926 this strain of White Odessa has been completely free from bunt, as compared with an average of 2.3 per cent of diseased heads produced in 1920 and 1921 under similar conditions. It is entirely possible that the original lot of seed of this variety was made up of two or more biotypes and that the plant used for this cross happened to be a completely resistant one.

Mackie supplied the writer with a quantity of inoculum which he had propagated on Little Club wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has grown this same collection on White Federation wheat at Davis. The inoculum, therefore, has been propagated from one original collection of bunt. This was done not primarily because it was suspected that physiologic forms of bunt existed, but because this offered an easy and definite source of spores. More recently, Faris (*4*), Rodenhiser and Stakman (*9*), Gaines (*6*), and Rodenhiser (*8*) have reported physiologic forms of this fungus. The fact that the same collection of bunt has been used continuously at Davis makes it quite certain that the same form or mixture of forms was employed. This is indicated also by the fairly constant way in which the parent material has reacted to inoculation with this collection.

The seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown during a period of three or four days, in order to avoid the effects of changing conditions of temperature and moisture. The nursery always was sown in a field in which no wheat had been grown during the previous year and which consequently was almost entirely free from volunteer plants.

At harvest time the plants in each row were pulled and separated into two piles, one bunt free and the other bunted. The total number of plants and the number of bunted plants were recorded and the percentage of bunted plants was calculated from these. A plant was classified as bunted if it showed even a trace of the disease.

EXPERIMENTAL RESULTS

The cross White Odessa × White Federation was made in 1925 and the F_1 generation was grown the following year. F_1 seeds were not inoculated because of the small number available.

Some of the F_2 seeds were treated with copper carbonate to protect them from bunt infection and thus insure a supply of seeds for the F_3 generation. The other F_2 seeds were inoculated in order to get some indication as to the number of factors present and to find out how many bunted plants to expect in F_3 rows of the same genotype.

F_2 data do not permit a Mendelian analysis, because some susceptible plants escape infection. Even in the most susceptible varieties rarely ever are 100 per cent of the plants diseased. Apparently the bunt-free plants in such a row merely have escaped infection, because they do not differ in resistance from the unselected variety. Data collected in F_2 are recorded in Table 1.

TABLE 1.—*Percentage of bunted plants in the parents and in the F_2 generation of the cross White Odessa × White Federation, when grown in the field at University Farm, Davis, Calif., in 1927*

Parent or cross	Number of plants		Percentage of bunted plants
	Total	Bunted	
White Odessa	258	0	0
White Federation	579	385	66.5
White Odessa × White Federation	422	94	22.3

There were 22.3 per cent of bunted plants in the F_2 of White Odessa × White Federation, which is very near the 25 per cent expected on the basis of a single dominant factor for resistance. Martin × White Federation produced 17.2 per cent of bunted plants in F_2 (*2*). It was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. In the case of White Odessa × White Federation either fewer susceptible plants escaped infection or more resistant and heterozygous plants became infected. That resistant and heterozygous plants occasionally may become infected will be shown later in this paper. F_2 data, then, indicate that White Odessa has a single dominant factor for resistance to bunt, and that heterozygous F_3 rows should contain an average of about 22 per cent of bunt.

In F_3 299 rod rows were grown from 299 F_2 plants which had been protected from bunt infection by seed treatment with copper carbonate. As already pointed out, the classification of F_2 plants on the basis of F_3 rows is more reliable than classification in F_2. The F_3 data are recorded in Table 2.

TABLE 2.—*Distribution of the parent and the F_3 rows of the cross White Odessa × White Federation into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1928*

Parent or cross	Distribution of rows by percentage classes of bunt infection																				Total number of rows
	0	<5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	
White Odessa	12																				12
White Federation											1		2	1	3		3	2			12
White Odessa × White Federation	57	13	11	21	33	47	29	12	5	2	8	0	4	3	6	12	15	16	4	1	299

The rows having from 0 to just less than 5 per cent of bunted plants were separated into those with none and those with some but less than 5 per cent of bunted plants, because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1. The numbers of rows under the three modes agree satisfactorily with the 1:2:1 ratio. There are 76 rows set off by the first minimum where 74.75 are expected. Between the first and second minima there are 162 rows where 149.5 are expected. This is a deviation of 12.5 ± 5.83, which is 2.1 times the probable error. From the second minimum on there are 61 rows where 74.75 are expected, a deviation of 13.75 ± 5.05, which is 2.7 times the probable error. The agreement is satisfactory, although not close. If the 8 rows with 45 to 50 per cent of bunt are considered as susceptible, the deviations all are within about one time the probable error. The

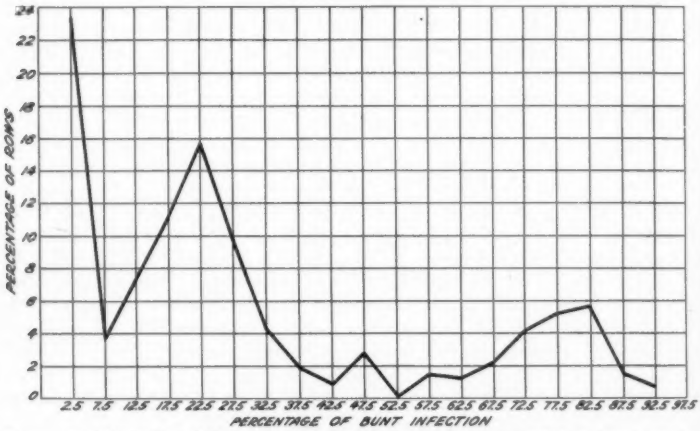

FIGURE 1.—Distribution of F_2 plants on the basis of F_3 rows of the cross White Odessa × White Federation into 5 per cent classes for bunt infection

minima should not be thought of as marking with absolute accuracy the divisions between phenotypes. However, it is interesting to note that in the curves for Martin × White Federation, Martin × Hard Federation, and White Odessa × White Federation the first minimum for each curve has fallen at 7.5 and the second one very near 50 per cent.

The 76 resistant rows were made up of 57 that were completely resistant, and 19 that contained a low percentage of bunt. Other data show that modifying factors may be responsible for the presence of a slight percentage of bunted plants in rows that were expected to be completely resistant like the resistant parent. In some cases the one or two diseased plants in a row may have been due to mechanical mixtures that occurred at the time of threshing or seeding.

The 162 F_3 rows from heterozygous F_2 plants averaged 23.5 per cent of bunted plants, as compared with 22.3 per cent in 10 F_2 rows. The F_3 rows contained from 7.5 to 47.5 per cent of bunted plants, as compared with 15 to 35 per cent in F_2 rows. Had as large a number of F_2 rows been grown, it is possible that similar extremes of bunt

infection would have been obtained. The seeds of bunt-free plants and partly bunted plants of F_2 row No. 355, which produced 15.8 per cent of bunted plants, and F_2 row No. 357, which produced 35.1 per cent, were saved and the F_3 was grown to see if these two extreme rows would come into line when the F_2 plants were classified according to bunted plants produced in F_3 rows. That there is satisfactory agreement with the single-factor hypothesis may be seen from the data in Table 3.

TABLE 3.—*Distribution of F_3 rows of the cross White Odessa × White Federation from F_2 rows Nos. 355 and 357 into 5 per cent classes for infected plants, when grown at Davis, Calif., in 1928*

5 per cent infection classes	Row No. 355					Row No. 357				
	Number of plants				Difference	Number of plants				Difference
	Observed by—		Expected	Difference	Probable error	Observed by—		Expected	Difference	Probable error
	Classes	Modes				Classes	Modes			
0	5	6	9.5	−3.5±1.80	1.94	15	17	14.25	+2.75±2.20	1.25
<5	1					2				
5–10										
10–15	2	20	19	+1±2.08	0.48	5	26	28.50	−2.50±2.55	0.98
15–20	5					7				
20–25	7					6				
25–30	5					4				
30–35	1					2				
35–40						2				
40–45										
45–50										
50–55										
55–60										
60–65	1	[a] 12	9.5	+2.5±1.80	1.39	1	[b] 14	14.25	−0.25±2.20	0.11
65–70	1					1				
70–75	2					1				
75–80	2					1				
80–85	2					1				
85–90	1					1				
90–95	1									
Total	38					57				

[a] Includes two plants that were completely diseased in F_2 and that therefore produced no good seed.
[b] Includes nine plants that were completely diseased in F_2 and that therefore produced no good seed.

If the plants that were completely smutted in F_2 are classed as susceptible and are added to the F_3 rows that are classed as susceptible, the agreement with the 1:2:1 ratio in both families is satisfactory. The greatest deviation is only 1.94 times the probable error.

In family No. 355 there were 2 completely bunted and 4 partly bunted plants in a row of 38 plants. Of the 4 partly bunted plants 3 proved to be homozygous susceptible and the other heterozygous. In addition, there were 7 susceptible plants which had escaped infection in F_2.

Family No. 357 produced 9 completely bunted and 11 partly bunted plants. Of the 11 partly bunted plants 3 were found to be susceptible, 6 heterozygous, and 2 resistant. There was one bunted head in F_2 on each of the 2 plants that proved to be resistant. Two susceptible plants had escaped infection.

There are several reasons, then, for the great differences in the percentage of bunted plants produced by heterozygous rows. In some

cases a large proportion of susceptible plants escape infection while in other rows only a few escape. In other cases apparently a rather large proportion of heterozygous plants become infected. Finally, in addition to mechanical mixture, there are resistant plants that become infected. The latter case is due fairly certainly to modifying factors. To what extent other differences are due to modifying factors is not known. Environmental influences may be active.

The group of susceptible rows, as indicated in Figure 1, produced an average of 75.6 ± 0.66 per cent of bunted plants as compared with 68.9 ± 2.15 per cent for the White Federation parent. The difference is 6.7 ± 2.25, which is 2.98 times the probable error. If the eight rows with 45 to 50 per cent bunt are considered as susceptible, the average for the susceptible group is 72.4, giving a difference of 3.5.

The segregation of F_2 plants, determined on the basis of the percentage of bunted plants in F_3 rows, shows that White Odessa differs from White Federation in one main dominant factor for resistance to bunt. The distribution of rows, as shown in Figure 1, is quite similar to that obtained for Martin × White Federation (*2*, *fig. 2*). Whether or not the factor for resistance in White Odessa is the same as the one in Martin is not known at present, but this point is being investigated.

DISCUSSION AND SUMMARY

In a previous publication (*2*) the writer has shown that Martin wheat differs from such susceptible varieties as White Federation and Hard Federation in one main dominant factor for resistance. He has also shown that Hussar (*2*, *3*) differs from such susceptible varieties in two main factors for resistance. One of these is the same factor as that found in Martin; the other allows bunt to develop on about half of the heterozygous plants. In the present paper data are presented which indicate that White Odessa differs from White Federation in one main factor for resistance, and that this factor is similar in its effect to the one found in Martin. Whether or not the factor in White Odessa is identical with that in Martin is not at present known.

Gaines (*5*) believes that resistance to bunt in such varieties as Martin, Hussar, and White Odessa probably is due to a large number of unit factors, the cumulative effect of which is to make the total result appear dominant, and that a lesser number would give a recessive effect.

McRostie (*7*), Aamodt (*1*), and others have shown the necessity of using known physiologic forms of the causal fungi in genetic studies of resistance to plant diseases. The same collection of bunt has been propagated and used continuously at Davis, but whether this is made up of more than one physiologic form is not known. A satisfactory set of differential hosts is not yet available for determining this point.

There may be other factors for resistance to bunt in Martin, Hussar, and White Odessa which would become apparent in the presence of other physiologic forms of this organism.

An attempt is being made to isolate as many different factors for resistance as possible. Once the different factors are isolated, their reaction to the different physiologic forms of bunt may be determined.

Some progress has been made in breeding bunt-resistant wheats. No doubt the effective breeding of bunt-resistant wheats in the

presence of physiologic strains of this disease will depend in some cases on bringing together two or more factors for resistance.

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ISOLATION AND PURIFICATION OF THE ALCOHOL-SOLUBLE PROTEIN (PROLAMIN) OCCURRING IN ENGLISH RYEGRASS (LOLIUM PERENNE) [1]

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INTRODUCTION

In spite of considerable difficulties encountered in dealing with plant proteins, their great significance as carriers of the most important vital functions in plant, animal, and man, together with their high nutritive value, makes it well worth the time and labor to throw additional light on their occurrence as well as on their physical and chemical properties. So far as English ryegrass is concerned, a careful perusal of the literature reveals the fact that knowledge concerning its chemical constituents is extremely meager. At present all that is known is the analysis by Deetz (*6*)[2] of the fat, ash, and nitrogen content of ryegrass plants in various stages of their development, and the reports of Gautier (*7*) and Arnaud (*1*) concerning their chlorophyll and carotin content, respectively. When the work of Schulze and his collaborators (*30*) on some nitrogenous compounds of the ryegrass is also mentioned, all of the literature dealing with its chemical constituents appears to be exhausted so far as the writer is aware.

We are indebted to Osborne for most of our knowledge concerning the alcohol-soluble proteins in plants. From his numerous researches, of which a few only can be mentioned here, and partly from those of Ritthausen (*29*), it is known that gliadin is present in the seeds of wheat (*Triticum vulgare*) (*25*, *26*, *27*) and rye (*Secale cereale*) (*21*, *26*), hordein in barley (*Hordeum vulgare*) (*22*, *27*), zein in maize (*Zea mays*) (*23*), and that a prolamin not named by Osborne is present in the seed of oats (*Avena sativa*) (*20*). Johns and Brewster as well as Hoffman have also reported the occurrence of prolamins in the seeds of kafir (*Andropogon sorghum*) (*19*) and of rice [3] (*Oryza sativa*) (*10*), respectively. Somewhat later the discovery of a prolamin in English ryegrass was reported by Jodidi and Peklo (*17*). The work on the aleurone layer by Peklo (*28*) is of interest in this connection. Yet in the joint paper the proof of the occurrence of prolamin in the ryegrass was based exclusively on the estimation of the nitrogen that could be extracted from the ryegrass with dilute alcohol. The prolamin itself was not isolated and hence could not be purified, analyzed, characterized by its reactions, or otherwise identified. It is with these problems that the present paper is concerned.

METHODS AND RESULTS

The protein nitrogen was determined according to Stutzer's method (*31*) as applied by the writer (*12*, *13*, *14*, *15*, *16*, *18*) in previous investi-

[1] Received for publication Aug. 9, 1929; issued February, 1930.
[2] Italic numbers in parentheses refer to "Literature cited," p. 369.
[3] Only a trace of a protein soluble in hot alcohol was found in the rice kernel.

gations. Sulphur was estimated by oxidizing the substance with a mixture of potassium nitrate and potassium hydroxide and precipitating the resulting sulphuric acid as barium sulphate.

FIRST ISOLATION AND PURIFICATION OF THE PROLAMIN

Five hundred grams of English ryegrass flour was extracted with 2 liters of 70 per cent alcohol at 55° C. for 24 hours, a deep brownish-red extract resulting. The extraction mixture was then strained through a cloth at about 50° and the residue squeezed out dry in a screw press. The resulting meal was extracted again with 1½ liters of 70 per cent alcohol at 55° for another 24 hours, strained through cloth, and the residue squeezed out again. The second extract was of a light-yellow color. The two extracts were united, filtered clear, and concentrated in the water bath at low temperature. On the surface of the liquid thin, transparent, brittle, brownish-yellow films formed, which were readily soluble in dilute alcohol, while there settled on the bottom of the dish a jellylike viscid mass which could be drawn to long threads or sheets. The substance thus concentrated was allowed to cool overnight. The following morning an attempt to filter the cooled residue through a filter on a Büchner funnel with suction failed completely. Filtration was extremely slow even through cheesecloth on a Büchner funnel. For this reason the mother liquor, which had a brown color due in part to anthocyanin as shown by its reactions, was decanted as much as possible and the residue washed with some water, after which it was centrifuged. The cake-like substance which remained in the centrifuge tubes was apparently quite impure.

The cakes were next treated in a flask with anhydrous ether for a period of 4 hours, the flask being shaken frequently. The ether, which assumed a yellow color due to the extracted fat, was decanted and discarded. The resulting cakes were then minced and treated with about 500 c. c. of absolute alcohol for 24 hours, the flask being shaken from time to time. The alcohol assumed a pretty wine-red color due to the coloring matter and the fat taken up, while the substance itself became lighter in color. The whole was then filtered with suction through a hardened filter paper on a Büchner funnel. The residue, which remained on the Büchner, was transferred to a flask and about 500 c. c. of fresh absolute alcohol was added; the mixture stood at room temperature for 36 hours, the flask being shaken occasionally. Despite the long treatment the alcohol assumed only a light-yellow color. However, when the treatment was continued for six hours more at 55° C. the alcohol assumed a reddish-yellow color, showing that at the higher temperature more of the pigment was extracted by the alcohol. The whole was now filtered through a hardened filter on a Büchner funnel, and since the alcoholic filtrate did not contain any protein, as shown by its failure to give a precipitate on the addition of much water and some sodium chloride, it was discarded. The residue on the Büchner funnel was then ground finely and treated three more times with absolute alcohol at 55° for periods of 24 hours each, when the alcohol failed to take up the pigment to any appreciable degree. The final residue on the Büchner was then extracted with boiling anhydrous ether in a Soxhlet apparatus for five hours, the substance, covered with ether, being allowed to remain in the apparatus overnight. The following morning the

ether, which had assumed a slightly yellow color, was siphoned off by means of the Soxhlet apparatus and discarded. This was followed by two more ether extractions effected in the manner just described, the last ether extract being colorless. The prolamin thus obtained had a grayish-yellow color and weighed 14.3 gm., equivalent to 3.04 per cent calculated on the basis of the oven-dried ryegrass, when dried to practically constant weight during three days in a vacuum over sulphuric acid. This substance, preparation 1, when analyzed was found to contain 4.60 per cent of moisture, 14.16 of nitrogen, and 0.49 of ash, calculated on a basis of the oven-dried prolamin.

TECHNIC USED IN MAKING THREE SUBSEQUENT PREPARATIONS

Another portion of 500 gm. of ryegrass meal was extracted with 2 liters of 70 per cent alcohol at 55° C. for 24 hours, which was followed by the extraction of the filtered residual meal with 1.5 liters of alcohol of the same strength, the operations having been effected as described for preparation 1. The two extracts were united, filtered clear, and concentrated in vacuo, at about 56° to 58°, to two-thirds the original volume, after which the substance was transferred to a porcelain dish and the evaporation continued on the water bath. The supernatant liquid, which was now decanted from the more or less solid protein, was further concentrated in a vacuum and, on cooling, centrifuged. A little more of the protein was deposited on the bottom of the centrifuge tubes. The supernatant liquid of the centrifuge tubes was again concentrated under reduced pressure, yielding another small portion of the protein. Both portions were added to the bulk of the protein, which was now treated with anhydrous ether at room temperature for 36 hours. The ether, which assumed a rich-yellow color, was filtered off and discarded. The solid material which remained on the Büchner funnel was, while still moist, cut up into small pieces, with scissors, and treated with ether for another 24 hours. The ether which had a light-yellow color was filtered off and discarded. This was followed by four treatments of the protein with fresh portions of absolute alcohol at 55° for periods ranging from 24 to 72 hours until the alcohol practically failed to take up coloring matter and the protein completely solidified. Finally the substance was extracted, first with anhydrous ether at room temperature, and then with boiling anhydrous ether in a Soxhlet apparatus for 10 hours. The ether, which appeared almost colorless, was siphoned off and discarded. The residue was preparation 2. From the data given subsequently it will be seen that preparation 2, of which a larger yield was obtained, has a lower nitrogen content than preparation 1. This is undoubtedly due to the fact that the former contains impure protein which was recovered from residual liquids.

A third portion of 500 gm. of ryegrass meal was extracted twice with dilute alcohol as outlined above. The two united and filtered extracts were concentrated under reduced pressure (748 mm. vacuum at 56° to 58° C.). The protein which had separated was purified by repeated treatments with ether, followed by several treatments with absolute alcohol at 55° until the protein had completely solidified. This was again followed by treatments with absolute ether, both at room temperature and at the boiling point of the ether. The prolamin thus purified had a yellow color and was dried in a vacuum over sulphuric acid for 72 hours. This constituted preparation 3.

Still another portion of 500 gm. of ryegrass meal was extracted twice with 70 per cent alcohol at 55° C., with the difference that the first and second extractions lasted 48 to 72 hours, respectively, instead of 24 hours each as in the previous extractions. The separation of the prolamin and its repeated purification, first with anhydrous ether, then with absolute alcohol, and again with anhydrous ether, at room temperature and at the boiling point of ether was effected essentially as outlined in the previous extractions. The final prolamin, on being dried in a vacuum over sulphuric acid for 48 hours, had a grayish-yellow color, and was labeled preparation 4.

The following analytical results were obtained: Preparation 2, which had a yellow color and weighed 18.4 gm. (equivalent to a yield of 3.91 per cent calculated on a basis of the oven-dried ryegrass), had 4.06 per cent moisture, 12.58 per cent nitrogen, and 0.69 per cent ash. Preparation 3, which weighed 14.6 gm. (equivalent to a yield of 3.11 per cent), contained 4.33 per cent of moisture, 14.18 per cent of nitrogen and 0.74 per cent of ash, calculated on a basis of the oven-dried prolamin. Preparation 4, which weighed 13.5 gm. (equivalent to a yield of 2.87 per cent), analyzed as follows: Moisture, 5.29 per cent; nitrogen, 13.96 per cent; ash, 0.83 per cent, calculated on a basis of the water-free protein.

It may be of interest to compare the yields of prolamin obtained from English ryegrass with those from cereal seeds. The data in Table 1 show that the yield of prolamin (provisionally called loliin) from ryegrass was, on the average, higher than the prolamin yield from Canadian and Oregon white winter wheat, but lower than that from South Dakota red spring wheat, and from rye, barley, and maize.

TABLE 1.—*Yield of prolamin obtained from ryegrass and cereal seeds*

Name of prolamin	Source of prolamin	Yield of prolamin calculated on the basis of—		Reference
		Air-dry seed	Oven-dried seed	
		Per cent	*Per cent*	
Gliadin	Canadian white winter wheat	2.74		Osborne (*24, p. 111*).
Do	Oregon white winter wheat	2.85		Do.
Do	South Dakota red spring wheat	8.15		Do.
Do	Rye	3.93		Osborne (*21, p. 439*).
Hordein	Barley	3.87–4.04		Osborne (*22, p. 564*).
Zein	Maize	5.00		Osborne (*23, p. 529*).
Loliin, preparation 1	English ryegrass	2.86	3.04	Jodidi, this paper.
Loliin, preparation 2	do	3.68	3.91	Do.
Loliin, preparation 3	do	2.92	3.11	Do.
Loliin, preparation 4	do	2.70	2.87	Do.

FURTHER PURIFICATION OF THE COMBINED PREPARATIONS

Since the four preparations described above did not differ materially from one another (the range of nitrogen content being from 12.58 to 14.18 per cent and the average 13.72 per cent), it was decided to unite them for the purpose of further purification. The principal impurities recognized were inorganic salts, especially chlorides, and carbohydrates. Therefore, the substance was washed with distilled water on a Büchner funnel provided with a hardened filter paper until the wash water gave but a faint reaction for chlorine, while the

reaction for carbohydrates was also slightly positive. The chocolate-colored residue which remained on the Büchner funnel was minced finely in some absolute alcohol, transferred to a flask to which 1 liter of absolute alcohol was added, and allowed to stand for three days at 55° C. The alcohol assumed a wine-red color. The whole was filtered on a Büchner with suction and washed with absolute alcohol. The residue on the Büchner funnel was again treated with absolute alcohol in the manner just described, after which it was treated with anhydrous ether for several days, the flask being shaken occasionally. On filtering off the ether, the residue, which had a brownish-gray color, was dried in a vacuum over sulphuric acid at room temperature until practically constant weight was obtained. This substance (preparation 5) was analyzed, with the following result: Moisture, 4.88 per cent; nitrogen, 14.30 per cent; ash, 0.80 per cent, calculated to the oven-dried substance.

In order further to purify the protein obtained, the following procedure was adopted: The substance was extracted with 1 liter of 70 per cent alcohol at 57° C. for 24 hours, the flask being shaken occasionally. The brownish-red solution obtained was filtered through a Büchner funnel provided with one soft and one hardened filter paper. There remained on the Büchner funnel a dark-brown quivering jelly with the following properties: When it contained a considerable proportion of water it would quiver as genuine jellies ordinarily do. However, when a part of the water was removed by suction and the jelly became fairly dry in the air overnight it formed a reddish-brown quite elastic cake not unlike rubber. When the jelly became perfectly dry in the air it turned hard and brittle and could be pulverized. It did not stick to either porcelain or paper. The filtered extract was concentrated in a vacuum at 57° to a volume of about 300 c. c. This was poured in a fine stream into 3 liters of ice-cold water. A few cubic centimeters of a concentrated sodium chloride solution was added, which caused a precipitate to appear. The following morning the yellowish-gray precipitate found on the bottom of the beaker was separated from the supernatant liquid by filtration and washed thoroughly with water. The precipitate was now dissolved in 70 per cent alcohol at 57°, a dark wine-red extract resulting. It was filtered clear, concentrated in vacuo to about 250 c. c., and poured in a fine stream into about 2 liters of absolute alcohol. The following morning the precipitated protein was separated from the supernatant liquid by decantation, thrice dehydrated with absolute alcohol, and finally treated with anhydrous ether as already described. The substance (preparation 6) was then separated from the colorless ether by filtration and dried in a vacuum over sulphuric acid for three days.

The dark-brown quivering jelly which had remained on the Büchner funnel from the extract just described was extracted six more times; the concentrated extracts were purified by pouring into large volumes of water and absolute alcohol, respectively, then dehydrated with absolute alcohol, macerated with anhydrous ether, and finally dried in a vacuum over sulphuric acid. These operations were carried out essentially as outlined in describing the first extract. The results obtained are summarized in Table 2.

TABLE 2.—*Analyses of the prolamin preparations obtained from extracts 1 to 7*

Extract No.	Preparation No.	Yield of prolamin	Moisture [1]	Nitrogen [1]	Ash [1]
		Grams	*Per cent*	*Per cent*	*Per cent*
1	6	3.3	3.98	14.46	0.44
2	7	4.9	3.91	14.61	.34
3	8	3.5	3.94	14.55	.73
4	9	2.1	3.92	15.02	.99
5	10	2.1	3.90	15.01	.70
6	11	2.1	4.66	15.41	.39
7	12	.9	4.16	15.15	.47

[1] Calculated on the basis of the oven-dried protein.

COAGULATION AND COMPOSITION OF THE PROLAMIN

It became evident from the extractions made that all of the prolamin originally obtained from the English ryegrass meal could not be extracted by digestion with 70 per cent alcohol at 57° C. For this reason different temperatures and different concentrations of alcohol were tried, but with negative results.

The impure prolamin as procured from 2,000 gm. of ryegrass meal was extracted at 55° to 57° with various fresh portions of 70 per cent alcohol successively in the expectation that most if not all of the prolamin would dissolve in that solvent. However, since the various extractions removed but a part of the prolamin, and the quantities obtained, or successive extracts, decreased gradually, it became evident that through the various operations incidental to the purification, especially through the repeated digestions at an elevated temperature, the prolamin acquired the property of becoming difficultly soluble or even insoluble (coagulated). A like behavior was observed by Osborne and by Chittenden and Osborne in their isolation of the prolamins from oats (*20*) and maize (*4*, *5*).

The preparations thus far obtained were evidently still impure, judging from their comparatively low nitrogen content. This may have been due partly to the fact that in those preparations an endeavor was also made to recover the protein out of the solutions nearly quantitatively. Therefore, it was decided in the next preparations to consider quality only and not to attempt to obtain large yields. The principal change made in procedure consisted in obtaining the next preparations by fractional separation. The analysis of the various preparations so made are summarized in Table 3.

TABLE 3.—*Percentage composition of various prolamin preparations (on a moisture and ash free basis)*

Item	Soluble prolamin preparations					Insoluble prolamin preparations					
	No. 13	No. 14	No. 15	No. 16	No. 17	No. 18	No. 19	No. 20	No. 21	No. 22	Average
Carbon	53.72	53.69	52.98	53.84	53.62	53.61	53.74	54.21	54.14	53.55	53.71
Hydrogen	6.61	6.93	6.58	6.64	6.86	6.40	6.79	6.58	6.64	6.59	6.66
Nitrogen	15.72	16.04	15.82	15.65	15.97	16.01	15.61	15.68	15.80	15.71	15.80
Sulphur	1.25	1.09	1.32	1.28	1.48	1.52	1.47	1.31	1.65	1.79	1.42
Oxygen	22.70	22.25	23.30	22.59	22.07	22.46	22.39	22.22	21.77	22.36	22.41
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

In reviewing Table 3 it will be seen that there are no essential differences between the soluble and insoluble (coagulated) prolamin preparations as far as their percentage composition is concerned. In other words, through the repeated heatings incidental to purification the prolamin underwent no changes other than to become insoluble. Similar observations were made by Osborne in his work on oats (*20*), as well as by Chittenden and Osborne in their work on maize (*4, 5*). In order to have a better idea of the significance of the figures given in Table 3, the average composition of the prolamin from English ryegrass is presented in Table 4, together with the average composition of the prolamins from the various cereals.

TABLE 4.—*Average percentage composition of the different prolamins*

Item	Zein (from maize)	Kafirin (from kafir)	Hordein (from barley)	Gliadin		Prolamin		
				From wheat	From rye	From oats [1]	From oats [2]	From ryegrass
Carbon	55.23	55.19	54.29	52.72	52.75	53.08	53.70	53.71
Hydrogen	7.26	7.36	6.80	6.86	6.84	6.91	7.00	6.66
Nitrogen	16.13	16.44	17.21	17.66	17.72	16.39	15.71	15.80
Sulphur	.60	.60	.83	1.14	1.21	2.26	1.76	1.42
Oxygen	20.78	20.41	20.87	21.62	21.48	21.36	21.83	22.41
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] Average of preparations 7 and 8. [2] Average of preparations 9, 10, 11, and 12.

THE NAME LOLIIN SUGGESTED FOR THE NEW PROLAMIN

Examination of Table 4 reveals the fact that, with the exception of the prolamins from wheat and rye (gliadin), which are apparently identical, all other prolamins differ more or less from one another in their chemical composition, a fact which has properly led to different individual names for the various prolamins. It further is evident that the prolamin from English ryegrass seemingly differs in its composition from all other prolamins, including gliadin, being nearest to the prolamin from oats, from which it differs, however, in several points. This being the case, the name "loliin" is proposed for this apparently new alcohol-soluble protein. The derivation of this name, from *Lolium perenne*, is fully analogous with that of the names of other well-known prolamins, such as hordein from *Hordeum vulgare* and zein from *Zea mays*. It is quite true that the name loliin has already been proposed for a volatile alkaloid (*2, 3*) supposedly occurring in the fungus of *Lolium temulentum*. Hofmeister (*11*), however, has proved that the alkaloid in question does not exist. Instead, he has shown the alkaloid temulin to occur in *L. temulentum*. Therefore, the use of the name loliin appears to be justified and fitting for the newly isolated protein.

NITROGEN DISTRIBUTION IN LOLIIN

For a further characterization of the isolated loliin, a study of its nitrogen distribution was made according to the method of Hausmann (*8, 9*), as modified by Osborne and Harris (*27*), and later adapted by Jodidi and Moulton (*16*). The results obtained are summarized in Table 5. For the sake of comparison the nitrogen distribution of several other prolamins, as reported by Osborne, also is given in the table.

TABLE 5.—*Nitrogen distribution of the various prolamins*

No.	Prolamin	Amide nitrogen [1]	Humin nitrogen [1]	Diamino nitrogen [1]	Monoamino nitrogen [1]	Remarks
		Per cent	*Per cent*	*Per cent*	*Per cent*	
1	Gliadin (from wheat)	4.33	0.14	0.98	12.21	
2	Hordein (from barley)	4.01	.23	.77	12.04	
3	Zein (from maize)	2.97	.16	.49	12.51	
4	Loliin (soluble, from English ryegrass)	3.32	.58	.50	11.16	Boiled with 20 per cent HCl in a glycerine bath for 12 hours.
5	do	3.34	.50	.49	10.57	Boiled with 25 per cent H_2SO_4 with Bunsen burner for 24 hours.
6	do	3.24	.55	.40	11.12	Treated like No. 4.
7	Loliin (insoluble, from English ryegrass)	3.27	.41	.58	11.00	Treated like No. 5.
8	do	3.22	.50	.48	10.84	Treated like No. 4.
9	do	3.24	.50	.66	10.88	Treated like No. 5.

[1] On the basis of the oven-dried prolamin.

It is readily seen in Table 5 that the amide nitrogen content of loliin is lower than that of gliadin and hordein, but higher than that of zein. While the humin nitrogen content of loliin is higher than that of gliadin, hordein, and zein, the reverse is true of the monoamino nitrogen, while the diamino nitrogen of loliin is about equal to that of zein but lower than that of gliadin and hordein. When preparations Nos. 4 to 6, representing the soluble modification of loliin, are compared with Nos. 7 to 9, representing the insoluble modification, it will be seen that the nitrogen distribution in both is practically identical. This shows, as in the case of their identical ultimate composition, that loliin, through repeated heating incidental to its purification, underwent no chemical changes. It may be mentioned here that the ryegrass used was not quite free from chaff, and, despite considerable efforts, it has been impossible to obtain loliin in a state of perfect purity, the final preparations always containing some coloring matter and perhaps other impurities. The next step to be undertaken by this laboratory will be the preparation of the highly purified protein in large quantity in order that it may be hydrolyzed into the individual amino acids of which it is composed.

SUMMARY

The alcohol-soluble protein occurring in English ryegrass was isolated for the first time, and its purification, analysis, and properties are described. It has been named loliin, the derivation of this name being analogous to that of the names of other well-known prolamins, such as hordein and zein. During repeated alcoholic treatments at elevated temperatures, incidental to the purification, a considerable portion of the prolamin becomes insoluble. The soluble and insoluble portions of the prolamin do not appear, however, to differ from each other chemically, since they have practically the same ultimate composition as well as the same nitrogen distribution in their respective molecules.

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THRESHER INJURY A CAUSE OF BALDHEAD IN BEANS[1]

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INTRODUCTION

Baldhead of beans (*Phaseolus vulgaris* L.), sometimes called snakehead, for a number of years has attracted the attention of seed growers and farmers, who have observed on seedlings the partial or entire absence of the primary leaves and the growing tip. Such plants, remaining dwarfed, are soon completely hidden by the foliage of the surrounding plants, and the erroneous impression is often gained from later inspections of the field that the plants have recovered and developed normally. Concern would not be aroused about the disease until similar seedlings were observed in a later planting or during another season.

Little information is available as to the history of baldhead, although interviews with seedsmen and growers have revealed the fact that it has been known for a number of years and is apparently on the increase. Because of the economic importance of baldhead and numerous inquiries as to its control, investigations were undertaken to determine its cause. What appeared a priori to be a simple problem proved in the end to be somewhat difficult and complicated. At first thought one might reasonably conclude that this abnormality is caused by insects, fungi, or bacteria; but certain relationships of baldhead to farm operations and its appearance in disease-free seed suggested that it could not be attributed to these agencies alone.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Inspection of bean fields in various parts of the United States revealed the fact that baldhead occurs practically wherever the crop is grown. The loss from baldhead plants, which make a poor growth and during the entire season produce at best only one or two imperfectly filled pods, is about equivalent to the percentage of affected plants at germination. This percentage varies according to the variety, the method of handling the crop, and the season. As a matter of fact, the amount of baldhead of any susceptible variety may vary from one season to the next as much as 5 to 10 per cent. Some varieties, such as Bountiful,[3] Refugee, and Improved Kidney Wax, are very susceptible, percentages of 10 to 30 not being uncommon.

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[3] The variety names used in this paper are those employed by the trade.

SYMPTOMS

Baldhead is caused by three distinct agencies, each producing well-defined characteristics. One type, which is by far the most common, is, as will be shown later in this paper, the result of mechanical injury. (Fig. 1, A–D.) The second type is caused by bacteria (fig. 1, E–G), and the third by insects. Although baldhead caused by insects has been investigated by Hawley [4] and has received no attention during these studies, it will be discussed briefly elsewhere.

Mechanical injury and bacterial infection produce quite different symptoms. In the case of mechanical injury the plumule may be entirely absent (fig. 1, B–D) or only vestiges of it remain. The plumule may at times remain attached to the epicotyl (fig. 1, A), although it is fractured just beneath the primary leaves, thus rendering further development impossible. These symptoms stand in striking contrast to those resulting from bacterial attack, in which case the entire plumule may be more or less completely destroyed (fig. 1, G) or the primary leaves may be badly mutilated, the terminal bud often being destroyed by the organism so that only remnants of the vascular foliar tissue remain. (Fig. 1, E. F.) Sometimes, in the absence of the terminal bud, the epicotyl elongates, often attaining a length of one-eighth (fig. 1, B, D) to three-fourths (fig. 1, A, C) of an inch. At this stage the plant may die, but more often buds develop (fig. 1, A, B) in the axils of the cotyledons, resulting in a compact growth of several leaves and branches with short internodes.

EXPERIMENTAL PROCEDURE

MATERIAL STUDIED

No investigations have been made for the single purpose of determining the entire range of plants subject to baldhead. Reports have been received of its occurrence on the different field and snap varieties of *Phaseolus vulgaris* and on *P. lunatus* L., the two species to which the investigations have been largely restricted. These studies were originally undertaken for the purpose of determining the cause of baldhead, and in so doing a number of different varieties, but by no means all, have been employed. Snap-bean varieties appeared to be especially subject to the disease, and for that reason most of the investigations were limited to them, a few varieties of the field type being used for comparison. From a more extended study of the different varieties it was found that field beans, as a group, are more resistant than the snap-bean varieties. Lima beans were not at first brought within the scope of the investigation, but a single germination test of one variety demonstrated a percentage of baldhead about equivalent to the snap-bean average. In view of this fact, three other varieties of Lima beans were brought under observation, as well as the Blackeye cowpea (*Vigna sinensis* Endl.) and the tepary bean (*P. acutifolius* var. *latifolius* G. F. Freeman).

GERMINATION

Preliminary experiments indicated that the presence of baldhead could be determined at about the time when the primary leaves of

[4] HAWLEY, I. M. INSECTS AND OTHER ANIMAL PESTS INJURIOUS TO FIELD BEANS IN NEW YORK. N. Y. (Cornell) Agr. Sta. Mem. 55, p. 945–1037, illus. 1922.

normal plants had attained a length about equal to that of the cotyledons. Beans germinated well in the several different materials tried, such as sand, sphagnum, soil, and between blotting papers.

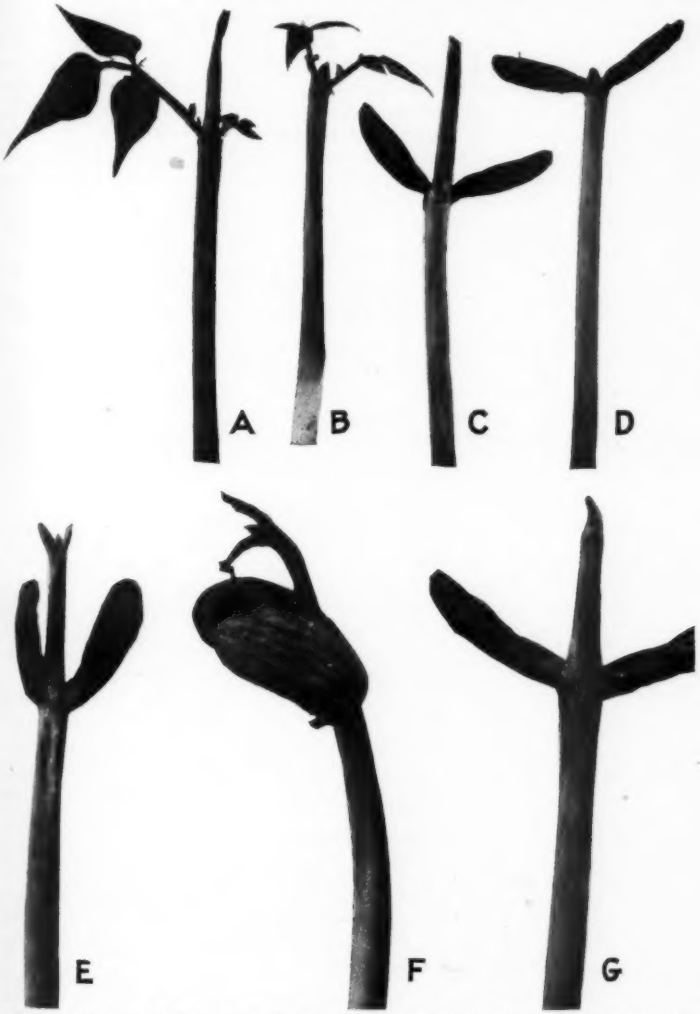

FIGURE 1.—Baldhead of beans as a result of: A–D, mechanical injury, which is characterized by the entire absence or only the vestiges of a plumule; E–G, injury caused by parasitic organisms, such as *Bacterium phaseoli*, which either destroy the entire plumule (G) or partially mutilate the primary leaves, leaving only ragged vestiges of the foliar tissue (E, F). E, F, G, slightly enlarged

The use of soil, sand, and sphagnum was objectionable because they were bulky or heavy and had to be sterilized each time before being used. Furthermore, they had no advantages over the following rapid and reliable method which was finally employed.

One hundred seeds, selected to insure apparent freedom from parasitic organisms, were disinfected for three minutes in a 1–1,000 solution of mercuric chloride and immediately rinsed in sterile water. The tests were conducted in moist chambers kept at laboratory temperature (22°–28° C.). In each moist chamber was placed a wire support, made of a square piece of heavy 4-mesh wire screen with the corners turned down about 1 inch; on this, blotting paper of proper size was laid. The seeds were then placed in the chamber so that they would not touch one another, and a second piece of blotting paper of the same size as the first was placed on top of them, after which all was thoroughly soaked with water. About one-fourth to one-half inch of water was poured into the moist chambers to maintain a relatively high humidity. Under these conditions most varieties germinated rather quickly, and at the end of five or six days radicles an inch or more in length had developed. At this stage the blotting papers were removed and the germinating beans were placed directly on the wire screen with the radicles inserted through the meshes and extending into the liquid below. Because of the paucity of food material in sterile distilled water, it was often replaced at this time by an equivalent quantity of Knop's solution. The presence of baldhead can be determined very effectively at this stage of development, but the seedlings were usually allowed to grow a few days longer until the primary leaves were one-fourth to one-half inch or even more in length, when a count was made of the affected plants.

RESULTS

EFFECT OF SEED MATURITY

In certain seed-growing districts of the West the season is comparatively short, so that varieties requiring a long season are sometimes frosted before all the seeds are mature—a contingency that was considered as having a possible causal relation to baldhead. With this in mind, some pods were gathered from plants in Virginia and in Colorado before the seeds were mature, and again from the same plants after the seeds had fully ripened. Although some of the immature seeds were badly shriveled, they germinated in most cases. The germination tests were made under similar conditions, with the result that there was no baldhead in either mature or immature seeds, showing that the stage of maturity is probably in no way associated with the malady.

INHERITABILITY

In 1926 seeds were collected from baldhead plants and threshed by hand, one part being tested for baldhead by germinating between blotting paper and the other planted in the field the following spring. No baldhead plants developed from any of these seeds. Second-generation seeds were also normal.

DESICCATION

Desiccation of the seeds, such as probably occurs in the arid regions of the West, resulting in injury to the plumule or epicotyl, was suggested as a possible cause of baldhead. An experiment was therefore designed to show whether such a relation existed. The varieties employed (Bountiful, Black Wax, and Kentucky Wonder Wax) contained a known percentage of baldhead and had been grown in Idaho and threshed by machine. Bountiful seeds, grown in Virginia,

threshed by hand, and containing no baldhead, were used for comparison. Desiccators containing concentrations of sulphuric acid previously calculated to give relative humidities of 1.5, 10.5, 21.5, 33, and 45 per cent were used. At the end of each month for four consecutive months 100 seeds of each lot were removed from each of the desiccators and germinated. The details of the results will not be given, inasmuch as the very slight increase in baldhead in those lots exposed to low relative humidities fell easily within the range of experimental error. The Kentucky Wonder Wax and Bountiful varieties, originally containing no baldhead, might, after an exposure to desiccation for four months, show 1 or 2 per cent of baldhead. From these results it seems that desiccation alone has very little if any effect on the production of baldhead.

MACHINE-THRESHED COMPARED WITH HAND-THRESHED BEANS

Snap and Field Beans

Germination tests of beans grown in California, Idaho, and Colorado, in comparison with seeds grown at the Arlington Experiment Farm, Rosslyn, Va., revealed some striking differences. The eastern seeds were harvested by hand and threshed either by hand or by pounding with a stick and contained little or no baldhead, whereas the seeds from the West contained a considerable amount. In seeking for an explanation of this difference, the probable variations in methods of handling were considered. The western seeds, obtained from commercial sources, were presumed to have been threshed by machine. This presumption gave a clue to the possible cause and suggested the desirability of a series of comparative germination tests. The earlier investigations were followed by similar tests in which the seeds were collected in such a way as to yield a direct comparison between machine-threshed and hand-threshed seeds. A number of varieties known to be subject to baldhead were chosen for study, and the seeds were picked and threshed by hand. Seeds from the same crop were obtained after machine threshing, and both were subjected to comparative germination tests. Hand-picked and hand-threshed seeds were also compared with seeds threshed by large and small threshers and by a flail.

The conclusions are drawn largely from the results of laboratory germination tests, but in 1927 and 1928 plantings were made from the same lots of seeds in the field in Colorado and in Virginia with the result that those varieties that gave a high percentage of baldhead in the laboratory generally gave a correspondingly high percentage when planted in the field. As might be expected, there was some variation due to the fact that insects and predatory animals, such as rodents, birds, etc., were responsible for the destruction of some plants and plant parts in the field. The evidence indicates, however, that the laboratory studies are a reliable guide as to what can be expected under field conditions.

The results given in Table 1, which includes all those varieties where a direct comparison of different methods of threshing could be made, show that threshing with either a large or a small machine in some cases produces a high percentage of baldhead. Flailing out the seeds causes some damage, but considerably less than either type of

machine threshing, while there were only two baldhead beans in all the hand-shelled beans tested.

TABLE 1.—*Variety, source of seed, and influence of method of threshing on the percentage of baldhead in beans*

Variety	Source	Percentage of baldhead resulting from threshing by—			
		Large machine	Small machine	Hand	Flail
Bountiful	Colorado	6.2		0	
Burpee's Stringless Green Pod	do	4		0	
Full Measure	do	13	1	0	
Improved Kidney Wax	do		6	0	
Stringless Green Refugee	do	8.2		0	
Davis White Wax	Idaho	19.2		0	
Full Measure	do	27.8		0	
Giant Stringless Green Pod	do	14.7		1	
Improved Kidney Wax	do	10.4		1	
Burpee's Stringless Green Pod	Wisconsin		18.5		0
Currie's Rust Proof	do		20		1
Early Stringless Refugee	do		23.3		5.5
Giant Stringless Green Pod	do		22.7		1.1
Hodson Wax	do		14.4		0
Improved Kidney Wax	do		20.6		3.6
Round Pod Kidney Wax	do		21		2.3
Sure Crop Wax	do		4	0	
Wardwell's Wax	do		12.7		0

For a comparative study of the percentage of baldhead in different lots of the same varieties of snap beans obtained from different sources, seeds were secured which were in most cases machine threshed. In a few lots shelling was done by hand; in others the information was not available, because the seeds were obtained from wholesale houses and from growers, but they are assumed to have been threshed by machine.

Table 2 shows that baldhead occurs in susceptible varieties of beans regardless of their source, if they are threshed by machine. Considerable variation occurs in seeds from different sources (Table 3), which is due probably not so much to any influence of the place of origin as to the condition of the pods when threshed. Some evidence has been collected which suggests that, if the vines and pods are damp when threshed the amount of baldhead is likely to be less.

LIMA BEANS

Like the snap and dry-shell types, Lima beans have been observed in some cases to develop a considerable percentage of baldhead in commercial field plantings, the fatalities often being so high as to arouse considerable concern on the part of growers and seedsmen. Only four varieties of Lima beans have been tested for baldhead, one of which (Henderson Bush Lima) was obtained from a seedsman in California and is definitely known to have been grown there. Three other varieties known to the trade as Fordhook Bush Lima, Emerald Isle Pole, and Sieva were purchased in Washington, D. C., from a seed dealer who had no information as to where the seed was grown or how it was threshed. It is not unlikely that they also came from California since most of the Lima-bean seed produced in this country is grown along the southern coast of California, and it is reasonably safe to assume that they were machine threshed.

TABLE 2.—*Percentage of baldhead in beans obtained from different sources when threshed by different methods*

Variety	Source	Percentage of baldhead resulting from threshing by—		
		Machine	Hand	Unknown method
Late Refugee	Idaho	18.5		
	Colorado	9.6		
Refugee, 1000-1	Virginia		0	
	Michigan			0
Early Refugee	do			5.1
	do			10.6
Bountiful	Idaho	23.8		
	Colorado	7.4		
	Virginia		0	
	Michigan			0
	do			2
	do			5
Red Valentine	Idaho	19.1		
	Colorado	14		
	Virginia		0	
	Michigan			5.5
	do			6
	do			4
Black Valentine	Idaho	15		
	Colorado	10.6		
Kentucky Wonder Wax	Virginia		0	
	Idaho	1.1		
Longfellow	California	3		
	Idaho	19.6		
Black Wax	Virginia		0	
	Idaho	22.1		
Black Wax Pencil Pod	California	8		
Prolific Black Wax	Colorado	9.4		
Great Northern	Idaho	1.1		
	do			0
Perry Marrow	New York			0
Well's Red Kidney	do			0
Michigan Pea Bean	Michigan			2
Robust	do			2
Improved Kidney Wax *	Wisconsin	24.5		
Red Kidney	California	25.5		
Bayo	do	11		
Cranberry	do	2		
California Pink	do	1		
Large White	do	8.5		
Genuine Small White	do	1.1		
Blue Pod Small White	do	2		
California Red	do	1.2		
Dutch Caseknife	do	18.9		
Lazy Wife	do	2.1		
Striped Creaseback	do	3		
Golden Cluster Wax	do	5.4		
King Mammoth Horticultural	do	12.2		
French Horticultural	Idaho	21.1		
Dwarf Horticultural	do	17.4		
Tennessee Green Pod	do	2.1		
Currie's Rust Proof	do	15.8		
Champion Bush	do	2		
Pinto	Colorado	1.5		
Red Mexican	Arizona			2.1

* Average of 6 lots, varying from 19 to 30 per cent baldhead.

TABLE 3.—*Summary of the percentage of baldhead in all varieties of beans from different sources when threshed by the different methods*

Source of seed	Number of seeds	Percentage of baldhead resulting from threshing by—		
		Machine	Flail	Hand
All sources	1,874			0.1
California	2,140	9.1		
Colorado	858	8.9		
Idaho	4,910	12.2		
Virginia	2,628		2.2	
Wisconsin	595	24.7		
Do	900	* 17.8		

* Threshed by small machine.

In germination tests similar to those conducted with the varieties of *Phaseolus vulgaris*, the following percentages of baldhead were obtained: Sieva, 2.17; Henderson Bush Lima, 11.11; Emerald Isle Pole, 13.67; and Fordhook Bush Lima, 15.95. These data show that Lima beans are liable to baldhead in percentages sufficiently large to result in considerable reduction in yield.

Cowpeas and Tepary Beans

There was no baldhead in the Blackeye cowpeas tested. In the tepary bean there was 6.88 per cent of baldhead.

Histology

An examination of the dry embryo or of the seed in a very early stage of germination, even before the integuments are ruptured, frequently reveals a fracture of the epicotyl just below the plumule, in which the latter may be partially or completely broken from the epicotyl. Figure 2, B, shows an early stage of germination of a seed in which the plumule is completely detached from the epicotyl. This should be compared with Figure 2, A, a normal seed germinated at the same time. Fractures such as those shown in Figure 2, B, are easy to detect by the unaided eye, but results indicated that baldhead sometimes occurs when the plumule and epicotyl appear to be normal, which suggested the possibility that an invisible injury to the tissues might occur. Consequently, permanent microscopic sections were made of normal and baldhead material. The seeds were germinated for a few days between blotting paper, and when the radicle had elongated slightly the cotyledons were carefully removed and the epicotyl with a portion of the hypocotyl was fixed in Carnoy's or formol-acetic-alcohol solution. After this they were carried through the usual processes of embedding, sectioning, staining, and mounting.

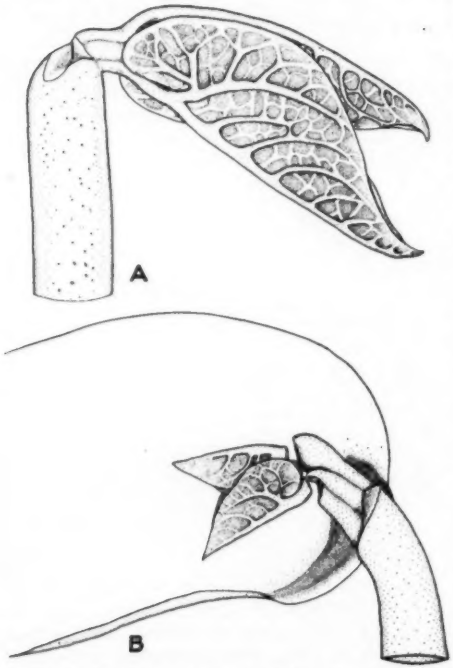

FIGURE 2.—Two bean embryos several days after germination: A, Normal embryo with both cotyledons removed; B, baldhead bean with one cotyledon removed to show the plumule broken off from the epicotyl. × 5

From among the many sections showing some type of injury, one was selected for the purpose of illustration. Figure 3, B, gives in outline a portion of this section, including a part of the two primary

leaves (*c*), the terminal bud (*d*), and the region just below the plumule, included between the two dotted parallel lines. A cursory examination of this region shows that the tissue of the epicotyl just between the primary leaves is torn at both margins, the tear extending slightly in the direction of the terminal bud and toward the center (fig. 3, B, *a*), partially dismembering the two primary leaves and terminal bud from the remainder of the epicotyl. Sometimes the tissue in the region below the terminal bud (fig. 3, B, *b*) is torn in one or more places, resulting in the production of irregularly shaped cavities, which vary in extent and number but which are usually in the general location shown by *b*. Figure 3, A, is a photomicrograph of the region shown between the dotted lines of Figure 3, B, and is of about the same magnification, while Figure 3, C, is a detailed drawing of the arrangement of the cells of this region at a like magnification.

The fractures in the epicotyledonary region differ somewhat in general appearance, but all produce the same result—the plumule fails to develop and finally becomes detached from the rest of the epicotyl. Sometimes the tear extends from the margin almost to the center of the epicotyl, and the plumule is held in place by only a few rows of cells.

RELATION OF DIAMETER OF EPICOTYL TO VARIETAL SUSCEPTIBILITY

An examination of the embryo of an ungerminated bean seed (fig. 4) with a dissecting microscope revealed a considerable diminution in the diameter of the epicotyl just below the plumule, where most of the fractures leading to the production of baldhead plants take place. This fact suggested the possibility that there might be some relationship between the diameter of the epicotyl and susceptibility to baldhead, those embryos with a small diameter in the epicotyledonary region presumably being more liable to injury from the threshing machine than those with larger epicotyls. Measurements then were made through the smallest diameter of the epicotyl of 25 seeds each of 18 varieties, which were selected to include 6 each of those which would be classed as very susceptible, susceptible, and highly resistant. Those varieties in which there was no baldhead, or not more than 1 or 2 per cent, were classified as immune or resistant. The embryo was carefully removed from the cotyledons, and measurements were made at a magnification of approximately 57 diameters, with a camera lucida being used to project the images upon a millimeter rule on the top of a table.

While one may not be justified in attaching any great importance to these data, the results show that the epicotyledonary diameter of the very susceptible, susceptible, and highly resistant groups average 0.45, 0.49, and 0.55 mm., respectively, the maximum being 22.2 per cent greater than the minimum. Of course it does not necessarily follow that the epicotyl with a small diameter is more easily broken than one with a large diameter, although, everything else being equal, such would probably be the case. The fact remains, at least, that the highest percentage of baldhead was found to occur in those varieties with the smallest diameter of the epicotyl.

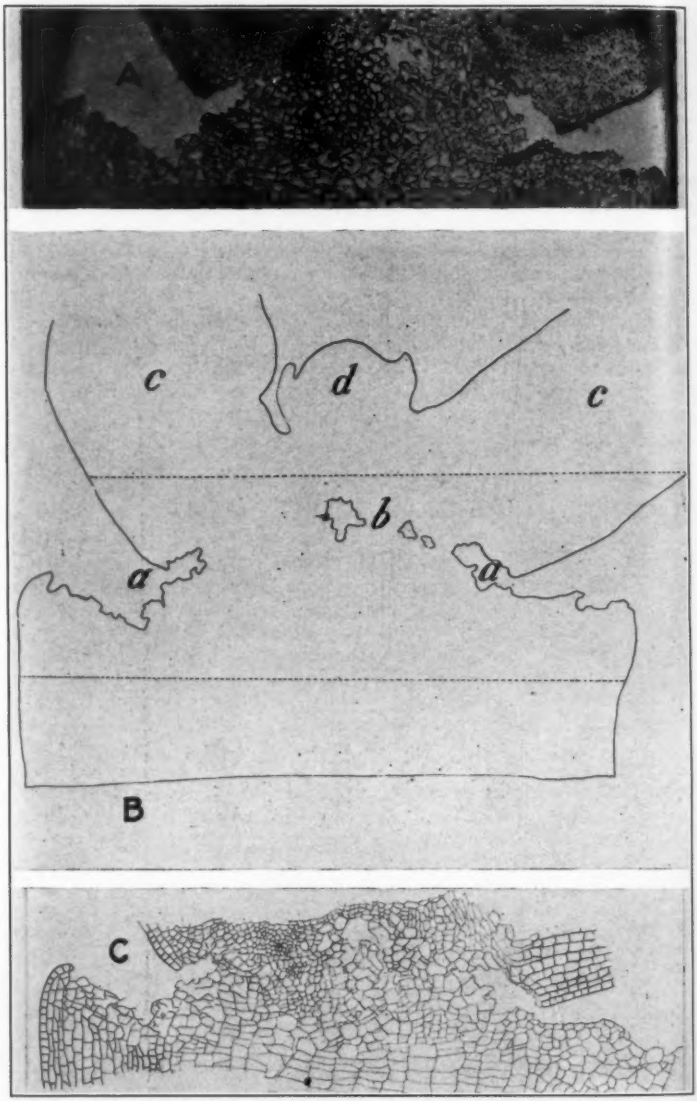

FIGURE 3.—Histology of baldhead: A, Photomicrograph of the area of a baldhead bean represented in B between the dotted lines; B, outline drawing, showing the primary leaves (*c*), the terminal bud (*d*), and the region where fractures occur (*a*, *b*); C, camera-lucida drawing, showing fracture and arrangement of cells in region covered by A. All about × 70

DISCUSSION

RELATION OF INSECTS TO BALDHEAD

According to Hawley,[5, 6] the seed-corn maggot (*Phorbia fusciceps* Zett.) causes from 50 to 75 per cent damage to beans in New York and other States as a result of abscissing the plumule, thus causing the plants to develop baldhead, or tunneling into the cotyledons while the seed is still in the ground. The insect also attacks the stem beneath the ground. This led Hawley to conclude that the entire loss attributed to the maggot was not due solely to the injury to the plumule, but in part to the injury to the stem.

BACTERIA AND FUNGI AS CAUSAL FACTORS IN BALDHEAD

While agreeing with Hawley [7] that the seed-corn maggot is responsible for most of the baldhead, Burkholder [8] claimed that *Bacterium*

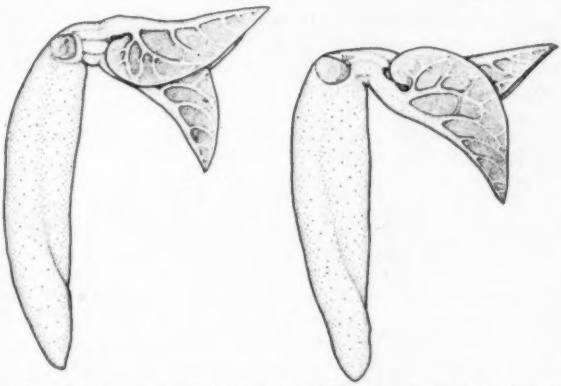

FIGURE 4.—Two embryos from dry beans, showing constriction in the epicotyl at the region w here fractures caused by the threshing machine take place. × 5

phaseoli EFS., as well as *Thielavia basicola* Zopf and a species of Rhizoctonia, may cause the same type of injury, although he gave no evidence to show that he produced the disease by inoculation with any of these organisms. His conclusions appear to be drawn entirely from the fact that *Bact. phaseoli*, *T. basicola*, and Rhizoctonia were isolated from baldhead plants. As additional proof Burkholder cited the results of planting blighted bean seeds in the greenhouse, 20 per cent of one lot producing baldhead while other plantings gave varying percentages.

In this connection, attention should be called to the fact that a high percentage of baldhead may occur in seed produced in regions of the West where bacterial blight does not occur, and attempts to isolate bacteria from specimens from those regions have always resulted in failure. Furthermore, it has been the writer's experience that in

[5] HAWLEY, I. M. Op. cit. (See footnote 4.)
[6] HAWLEY, I. M. SOME NOTES ON PHORBIA FUSCICEPS AS A BEAN PEST. Jour. Econ. Ent. 12: 203-205, illus. 1919.
[7] HAWLEY, I. M. Op. cit. (See footnote 6.)
[8] BURKHOLDER, W. H. THE BACTERIAL BLIGHT OF THE BEAN: A SYSTEMIC DISEASE. Phytopathology 11: [61]-69. 1921.

many cases isolations from the tips of baldhead plants from badly blighted seeds yield neither the blight nor the wilt organisms.

While the conclusions of Hawley and Burkholder are not questioned, the seed-corn maggot, *Bacterium phaseoli*, *Thielavia basicola*, and Rhizoctonia are not the sole causes of baldhead. The association of an organism, such as *Bact. phaseoli*, with baldhead does not constitute complete proof of a causal relationship. The bean-blight organism may gain entrance to the seed through the micropyle,[9] so that when germination starts, infection on the inner faces of the cotyledons is not uncommon. By the time the seedling has emerged from the soil the denuded epicotyl of already injured seeds may have attained a length varying from one-eighth to three-fourths of an inch; it is quite probable that it might have come in contact with blight lesions of the cotyledons and become infected or that it acts as a carrier only.

MECHANICAL INJURY DUE TO CONDITION OF THE SOIL

Many growers attribute baldhead to a mechanical injury of the epicotyl as the seedling pushes through the soil. Such accidents are supposed to be greatly increased when the penetration of the soil by the seedling is made more difficult by a dry, hard crust. However, it is not the epicotyl, protected as it is by the cotyledons, which is broken, but the hypocotyl, so that no further development takes place. This injury is sometimes mistaken for baldhead.

THRESHER INJURY

The occurrence of baldhead in bean seeds free from pathogenic organisms and in seeds germinated in the laboratory under conditions precluding infection by parasites was convincing evidence that there was another cause quite apart from insects, bacteria, and fungi. In seeds of almost every snap-bean variety grown in the West a considerable percentage of baldhead was found. This suggested a relationship between western-grown seed and the malady, but the results of investigations showed that there was nothing inherent in western-grown seed that would render it any more susceptible to baldhead than seed grown elsewhere. Commercial bean seed is raised in a comparatively few regions, but in all cases it is handled in the same manner. In comparing the methods of harvesting seed the commercial method of machine threshing is the only essential difference, and here apparently lies the causal factor to which is attributed more baldhead than to all the other factors combined—injury to the epicotyl while the seeds are being threshed.

The germination of hundreds of bean seeds showed that baldhead occurs in certain varieties threshed by machine, while seeds of the same lot shelled by hand gave none on germination by the same method. A microscopic examination of permanently mounted material showed that a fracture of the epicotyl of beans destined to become affected with baldhead occurred just beneath the plumule in machine-threshed seed. However, inasmuch as no such wound is found in hand-shelled beans, the evidence seems quite conclusive that the break is caused by the beans being violently struck by the teeth of the thresher cylinder or hurled against the teeth of the concave during threshing. Some varie-

[9] ZAUMEYER, W. J. SEED INFECTION BY BACTERIUM PHASEOLI. (Abstract) Phytopathology 19: 96. 1929.

ties are difficult to shell and can only be threshed by speeding up the cylinders, which might reasonably be expected to result in the splitting of many seeds and the fracturing of the epicotyl of others. The degree of desiccation of the seed at the time of threshing has been suggested as being somewhat correlated with baldhead; that is, if the beans were very dry when threshed, injury was more likely to result.

In general, the dry-shell field beans, as a group, seem to be less subject to baldhead than the snap beans, although the comparison has not been carried far enough to justify the drawing of positive conclusions. Out of 13 varieties of field beans investigated, only 3—the Red Kidney, Bayo, and Large White—all grown in California, showed a percentage of baldhead sufficiently large to be of any economic importance. On the other hand, a considerable percentage of baldhead occurred in most varieties of snap beans, but there are a few exceptions, among which may be mentioned the Kentucky Wonder Wax, Tennessee Green Pod, and Lazy Wife.

Several theories have been proposed to explain the general dissimilarity between the dry-shell and snap-bean varieties, but all are untenable or can be negated by several exceptions. The growers of snap beans for seed purposes generally agree that stringless varieties are more difficult to thresh than stringy ones and that the difficulty of threshing is increased if the vines are cut before the pods are entirely ripe. As a consequence the seeds must be released from the pods by speeding up the cylinders or by more nearly closing the concave, both of which may cause some splitting and cracking of the seeds. In threshing the stringless varieties the pods break crosswise at each side of the seed, leaving the bean incased in a segment of the pod. The stringy varieties, among which are the dry-shell field beans, have a large amount of xylem paralleling the dorsal and ventral sutures along which the pods split instead of breaking into segments when struck by the cylinder, thus releasing the beans without too violent treatment. While there seems to be some evidence that baldhead is more prevalent in the stringless varieties than in the stringy ones, the correlation is far from being perfect, since a high percentage of baldhead is sometimes found in some of the stringy varieties and a low percentage in certain lots of some of the stringless ones.

While the stringiness of the bean may partially explain the occurrence of baldhead, undoubtedly other factors, such as the maturity of the crop when harvested and the degree of curing and desiccation of the vine and of the bean itself when threshed, may play important rôles. Information acquired from reliable sources indicates that beans cut a little too green are difficult to thresh, due to the fact that the pods dry tightly about the beans, especially if weather conditions are unfavorable for curing.

Much of the snap-bean seed is grown in the arid regions of the West, where the annual rainfall alone is insufficient to grow a crop and water is supplied by irrigation several times during the growing season. After the beans have reached a certain stage of maturity, water is withheld and the crop is allowed to ripen. It sometimes happens that the ripening process is started too soon, or that an unexpected period of warm weather occurs, so that the beans are subject to drought conditions and ripen prematurely. Under such conditions the pods shrink and tighten about the seeds, rendering their release during threshing extremely difficult and causing them to break into 1-seeded

segments instead of cracking along the sutures. In the light of the evidence at hand, the indications are that not one but a combination of seasonal factors render the seed of certain varieties subject to injury by the threshing machine.

SUMMARY

Investigations have been made of baldhead in beans, a seedling abnormality in which the plumule is absent.

Germination tests of snap and field beans, together with histological studies of the epicotyledonary region of embryos from hand-threshed and machine-threshed beans, have shown that the epicotyl is fractured just below the plumule by the threshing machine. Baldhead rarely occurs in beans threshed by hand. Baldhead plants may develop buds in the axils of the cotyledons which may result in a few flowers and possibly one or two partially filled pods, but never a full yield.

The percentage of baldhead varies with the different varieties and ranges from 0 to 30 per cent. Among some of the snap-bean varieties 10 to 20 per cent is common. Baldhead occurs only to a very slight extent or not at all in many of the field or dry-shell beans studied.

The average diameter of the epicotyl in the embryonic stage was found to be less in snap beans susceptible to baldhead than in more resistant field beans.

BACTERIAL LEAF SPOT OF SQUASH[1]

By MARY K. BRYAN

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INTRODUCTION

Bacterial leaf spot of squash first came to the attention of the writer in 1925 when specimens were sent to E. F. Smith from Ithaca, N. Y., by C. D. Chupp, who wrote concerning the infected plants:

> The disease started in the center of a small planting of Hubbard squash and is spreading rather rapidly in all directions. It kills the foliage and seems to be very virulent.

In reply to a request for further information he wrote:

> The plants were not killed entirely, but many of the leaves were so badly blighted that the plants looked almost black. Apparently the bacterium must have been carried in the seed, since none of the Hubbard squash were grown in that vicinity, and so far as I know Hubbard squash had never been planted in that garden before.

It was at first suspected that this was a new host for *Bacterium lachrymans*, but the isolation of a yellow bacterium that was proved pathogenic to squash by inoculation disproved this theory. In a brief description of the organism [2] the name *Bact. cucurbitae* was proposed. A short account also appeared in the Yearbook of the United States Department of Agriculture.[3] The present paper is intended to give a more complete description of the pathogene and of its effect on its hosts.

GEOGRAPHICAL DISTRIBUTION

Since its discovery in New York, bacterial leaf spot of squash has been found in Maryland, the District of Columbia, Georgia, and South Carolina. In New York it has been reported on Hubbard squash only, but in Maryland it was found on Hubbard squash and Boston Marrow (*Cucurbita maxima*), pumpkin, and Golden Summer Crookneck squash (*C. pepo condensa*). In the District of Columbia, Georgia, and South Carolina it has been found only on Golden Summer Crookneck squash.

SYMPTOMATOLOGY

Lesions are confined chiefly to the leaves, but occasionally young stems and petioles are attacked. No infections have been found or produced by inoculation on fruits. Leaf spots are conspicuous, especially in the early stages, for the wide, bright-yellow halo that surrounds them.

Infections are first apparent two or three days after spray inoculation. At that time they show on the lower surface as minute water-soaked areas penetrating to the upper surface as indefinite yellow

[1] Received for publication Sept. 10, 1929; issued February, 1930.

[2] BRYAN, M. K. BACTERIAL LEAF SPOT ON HUBBARD SQUASH. Science (n. s.) 63: 165. 1926.

[3] BRYAN, M. K. SQUASH OF HUBBARD VARIETY ATTACKED BY NEW LEAF SPOT. U. S. Dept. Agr. Yearbook 1927: 599–600., illus. 1928.

areas. They enlarge rapidly, so that by the fourth or fifth day there is a definite round or angular, thin, brown, translucent center having on the upper surface a wide yellow halo (fig. 1) and on the lower surface either a water-soaked margin or no differentiation beyond the sunken spot. With further enlargement the spots are angular and restricted by the veins. Single isolated spots may reach a diameter of 6 or 7 mm.

Large dead areas are produced by the coalescing of crowded spots, as shown on the lower part of the inoculated leaf. (Pl. 1.) Here each spot is delimited by the veins which separate it from adjoining spots. The whole area is thin, dry, translucent, and mottled light and dark brown. The same patchwork effect is seen in the original material from Ithaca, N. Y. (Pl. 2.) Although papery, thin, and dry, the spots do not tear and drop out as in angular leaf spot of cucumber.

FIGURE 1.—Young infections on a Hubbard squash leaf from a hothouse plant. Collected seven days after spraying with *Bacterium cucurbitae*. × 1

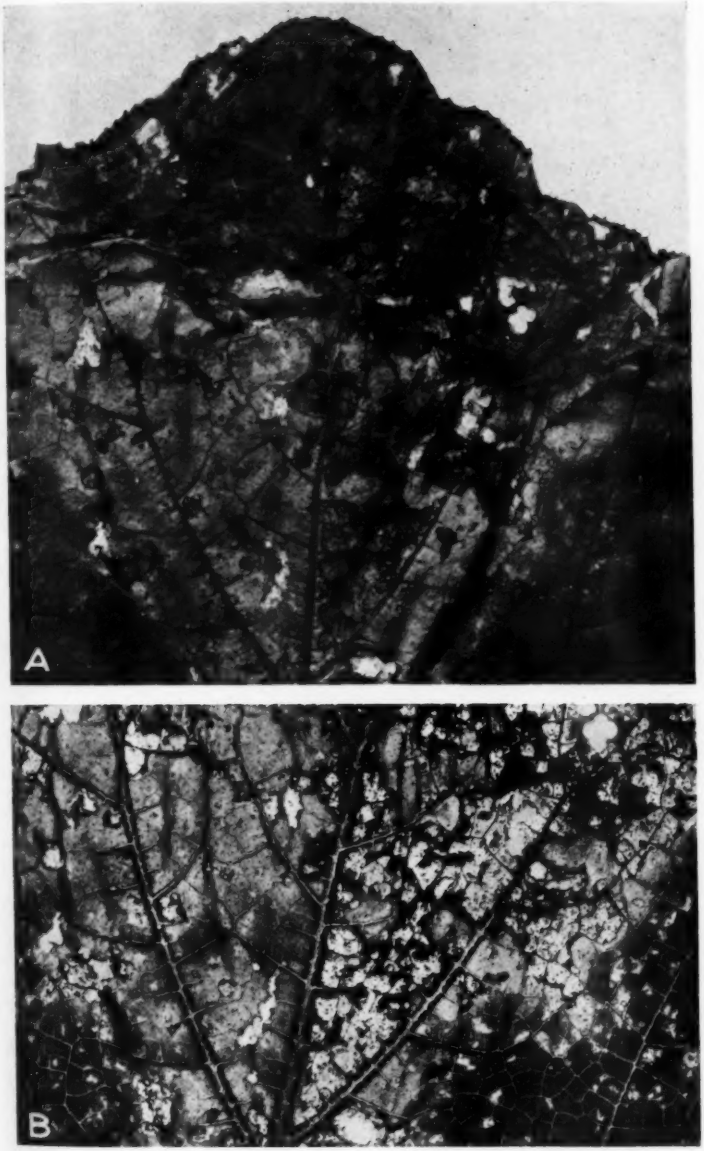
Large dead areas also form where injury to veins causes the shriveling of the tissues beyond. This is seen in the tip of the leaf shown in Plate 1, A. Spots and aggregates of spots may be distinguished within this dead, opaque area.

On shaded portions of leaves the spots may be dark gray instead of brown; the yellow halo is as conspicuous on such spots as on those exposed to direct sunlight.

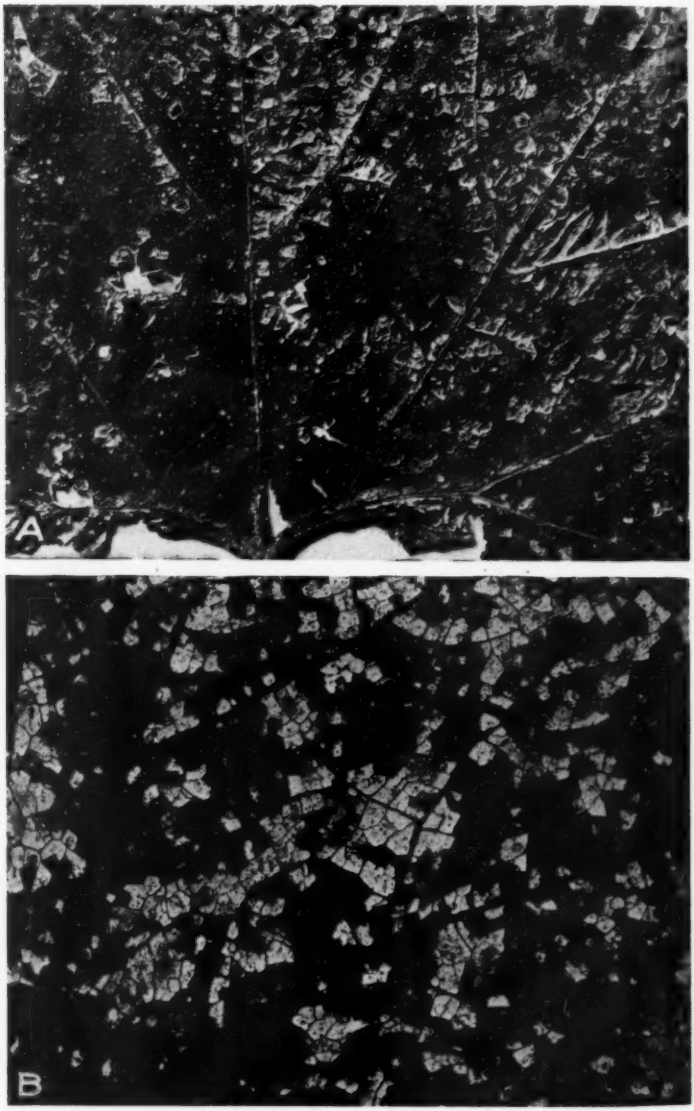
Occasionally young stems of summer squash are attacked (fig. 2) and cracking results. Very young plants may be killed by injury to the growing point when thus infected.

PATHOLOGICAL HISTOLOGY

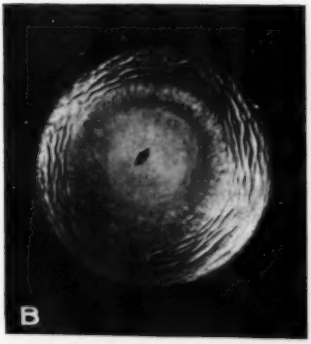
The bacteria enter the leaf by way of the stomata, which are very numerous on both leaf surfaces, especially on the lower one. Bacteria may be demonstrated in the substomatal chamber and penetrating half the thickness of the leaf on the second day (pl. 3, E), after inoculation by spraying. At that time the only outward indication of infection is a slightly water-soaked spot on the lower surface. By the fourth day bacteria have spread in all directions, filling the intercellular spaces of the loose parenchyma in the lower part of the leaf and wedging between the palisade cells to the upper epidermis. In stained sections cut at this stage no collapse has taken place. The cells of the infected area are turgid, but the cell walls in the invaded area take a deeper stain than those in the adjoining tissues. Very shortly after this stage the invaded tissues collapse. This collapse is much more evident on the lower surface than on the upper.

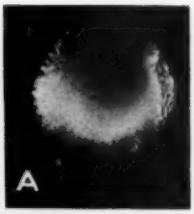


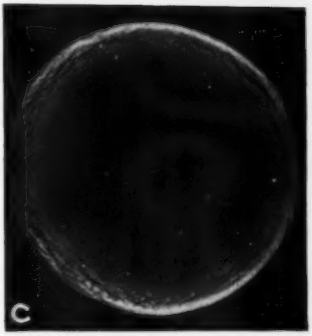
Secondary infections on a Hubbard squash leaf from a plant inoculated with *Bacterium cucurbitae*: A, Photographed by reflected light; B, by transmitted light. × ⅔

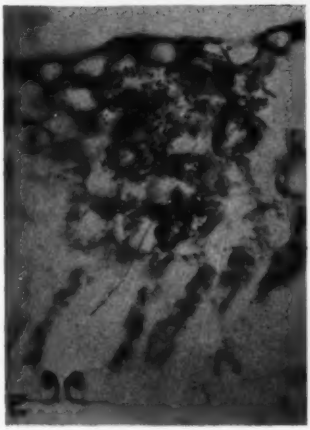


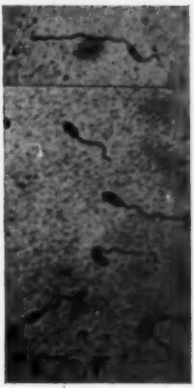
Dry leaf, showing natural infection by *Bacterium cucurbitae* on original Hubbard squash leaf from Ithaca, N. Y.: A, Photographed by reflected light; B, by transmitted light. × ⅔











A–C.—Agar-plate colonies of *Bacterium cucurbitae* taken by oblique transmitted light: A, 5 days old, × 5; B, 7 days old, × 5; C, 9 days old, × 7

D.—Milk culture, 4 weeks old

E.—Section of Hubbard squash leaf showing stomatal infection two days after inoculation by spraying with *Bacterium cucurbitae*. × 400

F.—*Bacterium cucurbitae*, showing flagella stained by Casares-Gil's stain. Note the extremely long flagellum on the uppermost rod. These occur frequently on this organism. × 940

Bacterial leaf spot should not be confused with the virus disease described as ring spot by Wingard.[4] In early stages of systemic ring spot on squash where the water-soaked or necrotic center is of pin-point size with a yellow halo, it might be difficult to distinguish the lesions from those of bacterial spot. Later stages could not be confused. On pumpkin, however, the spotting as shown in Wingard's[5] Figure 20 is very suggestive of bacterial spot, except that in the latter disease the spots do not follow the veins.

INOCULATIONS

Stomatal infections are readily produced on Hubbard squash by spraying the plants in inoculation cages with a suspension of *Bacterium cucurbitae*. From two to three days elapse between the time of inoculation and the first appearance of lesions. The youngest completely or partially expanded leaves are most susceptible.

Good infections have been obtained in the manner just described on all the varieties of the genus Cucurbita on which the disease occurred in the field and also on Italian summer squash, Early White Bush Scalloped squash, Large Field pumpkin, and Sugar Pie pumpkin. Several varieties of *Cucurbita moschata* (Striped Cutshaw, Winter Crookneck, and Large Cheese pumpkin) have been successfully inoculated. Cucumis species, such as cucumber (*C. sativus*) and muskmelon (*C. melo*), appear immune. Very minute infections were once produced on cucumber, but they did not develop beyond pin-point size. Good infections have been obtained on watermelon (*Citrullus vulgaris*). (Fig. 3.)

FIGURE 2.—Stem of young Golden Crookneck summer squash infected with *Bacterium cucurbitae*. ×1

Reisolations were made from spots on each of the successfully inoculated varieties, and the characteristic colonies thus obtained proved infectious on Hubbard squash.

[4] WINGARD, S. A. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. Jour. Agr. Research 37: 127–153, illus. 1928.
[5] WINGARD, S. A. Op. cit.

Seed inoculated with *Bacterium cucurbitae*, dried and planted, have yielded as high as 15 per cent infection, the disease sometimes showing first on the cotyledons and at other times on the first leaves.

Inoculations with *Bacterium cucurbitae* both by spraying on and pricking in the inoculum, on cotton, cabbage, string bean, Lima bean, and poppy, all of which are hosts of other yellow plant patho-

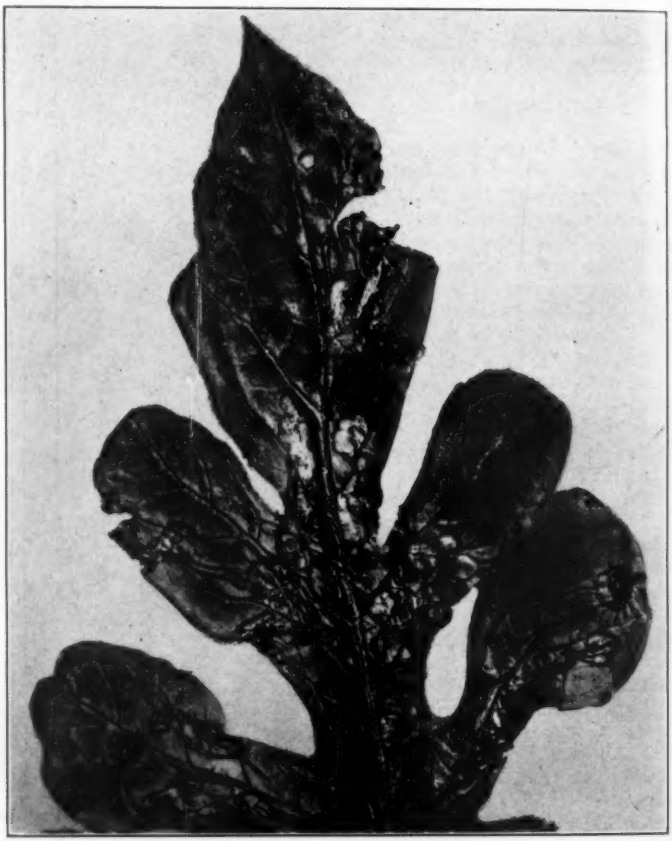


FIGURE 3.—Watermelon leaf five days after spraying with *Bacterium cucurbitae*. × 1¾

genes, have been entirely negative. Good infections resulted on Hubbard squash used as checks.

METHOD OF DISSEMINATION

The organism is apparently carried on the seed, but it does not appear to infect the soil. Although weather conditions favored infection, perfectly healthy Hubbard squash vines were grown on a plot in the District of Columbia where the disease had been very

severe the previous year. Further observations are needed to complete the evidence on these points. Spread in the field is facilitated by weather conditions, chiefly by rain. Potted plants of Hubbard squash, with several infected leaves each, set out in the ground early in July, developed vigorous healthy vines with no new infections during a long period of dry weather, but during a 10-day rain in August following this drought heavy infection spread rapidly through the patch and continued to spread with succeeding rains. Newly infected leaves appeared adjacent to old infections, and although squash bugs, cucumber beetles, and aphids were abundant on the vines, no infections were found where they fed on the leaves, at a distance from infected leaves. It would seem, therefore, that these insects do not spread the disease.

THE PATHOGENE

MORPHOLOGY

Bacterium cucurbitae is a short, polar-flagellate rod, 0.5μ to 1.3μ by 0.45μ to 0.6μ, usually with only one flagellum, which is frequently of unusual length. (Pl. 3, F.) It occurs singly, in pairs, or in short chains. Capsules are formed, but no spores.

REACTION TO STAINS

This organism stains readily with the usual bacterial stains, is definitely Gram negative, and is not acid fast.

CULTURAL CHARACTERS[6]

BEEF-AGAR PLATES.—Colonies on thinly sown plates are visible on the third day. They are less than 1 mm. in diameter, round, pale yellow, opalescent, and finely crosshatched or without markings. By the fifth day they are 3 or 4 mm. in diameter, showing by transmitted light an opaque center and a thinner margin, which may be either crosshatched or show concentric lines. (Pl. 3, A.) Some colonies are slightly umbonate. Later they may reach a diameter of 6 or 7 mm. and become decidedly umbonate. The concentric lines or crosshatching persists, and the opalescence remains very striking. (Pl. 3, C.) Sometimes fine radiating lines appear in the margin (pl. 3, A) or as a ring between the dense center and the thin margin. (Pl. 3, B.) The color, at first wax yellow, becomes mustard yellow.[7] The consistency is at first viscid and later butyrous. On plates 2 weeks old secondary growths of deeper color have formed in the colonies. Buried colonies are lenticular; bottom colonies thin, white, and granular with one or two concentric rings.

BEEF-AGAR SLANTS.—Growth is moderate, 2 to 6 mm. wide, mustard yellow, opalescent, and smooth with undulating margins. The consistency is viscid at first, but it becomes butyrous. Usually no crystals are formed, but occasionally a few small crystals appear just below the surface of the slant.

BEEF-EXTRACT AGAR.—Growth on beef-extract agar is deeper yellow, more restricted with less noticeable markings and less conspicuous opalescence than on beef-infusion agar.

BEEF BROTH.—Clouding is prompt but moderate. A rim of coarse pseudozoogloeae is formed, and in undisturbed cultures also a partial or complete pellicle of the same composition. Cultures 3 weeks old or more are clear or almost so and have a heavy yellow precipitate, which rises in a viscid swirl on shaking. Numerous small crystals lie in the precipitate.

POTATO-DEXTROSE AGAR.—On this medium growth is abundant, soon covering the entire surface with a heavy, butryous, pale-yellow layer of growth.

[6] Unless otherwise stated, all beef media were made with beef infusion and had a pH of 6.8 to 7.2.

[7] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912

POTATO CYLINDERS.—On steamed-potato cylinders growth is very heavy, butyrous, and bright yellow, covering the potato and filling the water with a dense yellow slime, eventually almost or completely submerging the potato, which shrinks with the destruction of the starch. Old cultures take on a more or less deep-brown tinge.

SYNTHETIC MEDIA.—In Cohn's solution growth is very feeble or absent. Very moderate growth occurs in Uschinsky's solution. In Fermi's solution cultures cloud moderately. In none of these media is there any rim, pellicle, precipitate, or fluorescence.

PHYSIOLOGY

LIQUEFACTION OF GELATIN.—In gelatin stabs at 20° C. liquefaction begins on the second day, is saucer shaped, becoming stratiform, and is only half complete in one month. At this stage there are an abundant yellow precipitate and a yellow rim, but the liquefied gelatin is practically clear. Liquefaction is complete in six to eight weeks.

LIQUEFACTION OF BLOOD SERUM.—Clearing of the serum begins on the fourth day, followed on the fifth by liquefaction, which extends throughout the slanted portion and slightly below; but neither process reaches the bottom of the tube.

FERMENTATION OF SUGARS.—The ability of the organism to ferment various carbohydrates was tested on peptone-free synthetic agar[8] with brom-cresol purple as an indicator. One per cent of each of the following was used: Sucrose, dextrose, lactose, maltose, galactose, levulose, glycerin, and mannit. Acid was formed from all except mannit, beginning on the seventh day in sucrose, dextrose, galactose, and maltose, and in nine days in lactose, glycerin, and levulose. The cultures never became acid throughout, the lower portions remaining pale purple.

When the same carbohydrates were tested in beef-extract agar with brom-cresol purple as indicator no acid was evident at any time; in the same beef-extract agar with the same carbohydrates and with phenol red as indicator, an alkaline reaction began within 24 hours and within 10 days had spread throughout the agar in all cultures.

No gas was formed in fermentation tubes from any of these carboyhdrates, nor was there any growth in the closed end.

HYDROLYSIS OF STARCH.—The starch of potato cylinders is almost completely destroyed and the shrunken cylinder submerged in the abundant yellow growth. On starch-agar plates a clear area 2 to 2.3 cm. wide around the streak of bacterial growth is produced within seven days.

RELATION TO FREE OXYGEN.—The organism is aerobic; it does not grow in the closed end of fermentation tubes and makes no growth in the lower half of agar stabs or in the lower part of shake-agar cultures.

NITRATE REDUCTION.—Nitrates are not reduced.

REACTIONS IN MILK.—No acid is formed in milk; peptonization sets in on the fifth or sixth day and is complete in eight days. Separation begins at about this time. At the end of three weeks the cultures present the appearance shown in Plate 3, D, with a heavy yellow rim above a layer of clear whey; below this is a heavy yellow precipitate on top of the soft curd, which has drawn away from the sides of the tube. Occasionally a few tyrosine crystals are formed. Reduction of methylene blue begins in three days and is complete in seven days. Reduction of litmus begins by the tenth day and is complete by the fourteenth day.

PRODUCTION OF AMMONIA.—A moderate amount of ammonia is produced in beef media.

PRODUCTION OF HYDROGEN SULPHIDE.—Hydrogen sulphide is produced in beef broth. Tests with strips of lead-acetate paper inserted in beef-broth cultures became a deep brown by the sixth day.

PRODUCTION OF INDOL.—No indol is produced.

TOLERATION OF SODIUM CHLORIDE.—Clouding is prompt and heavy in beef broth containing 1 and 2 per cent NaCl. It is retarded slightly in the presence of 3, 4, and 5 per cent. Growth in 5 per cent remains very moderate and contains many chains.

OPTIMUM pH FOR GROWTH.—The organism makes its best growth in pH 6.5 to 7.0. The limits for growth are pH 5.8 and pH 9.0.

TEMPERATURE RELATIONS.—The best growth takes place at 25° to 30° C., no growth occurs above 35°, and the thermal death point is 49°.

LONGEVITY ON CULTURE MEDIA.—Cultures of this organism are shorterlived on culture media than most pathogenes; even beef-agar stabs, when kept in the

[8] SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA . . . 48 p., illus. Geneva, N. Y. [1923.]

ice box, must be transferred every three or four months, as after six months without transfer most strains are dead.

TECHNICAL DESCRIPTION

Bacterium cucurbitae is a short rod 0.5 μ to 1.3 μ by 0.45 μ to 0.6 μ, with one polar flagellum, occurring singly, in pairs, or in short chains. It forms no spores, is not acid fast, and is Gram negative. On beef agar it forms round, yellow, opalescent colonies with internal striae. Its diastasic action is strong. It liquefies gelatin and blood serum slowly. Acid without gas is formed from sucrose, dextrose, lactose, maltose, galactose, levulose, and glycerin, but not from mannit. It does not form acid in milk, but reduces litmus and methylene blue in this medium. It does not reduce nitrates or form indol, but it does produce ammonia and hydrogen sulphide. Its optimum pH or growth is 6.5 to 7.0. Its optimum temperature is 25° to 30° C., its thermal death point being 49°.

SUMMARY

This paper describes a bacterial leaf spot which attacks summer and winter squashes and pumpkins. The disease can be transmitted to watermelon by inoculation, but it has not been found occurring on this plant in the field. It has not been found on cucumbers or muskmelons, and inoculations have failed to produce it on them.

The leaf spots are brown surrounded by a yellow halo, and they do not tear or drop out as do those of angular leaf spot of cucumbers. They often coalesce to form large dead areas.

The disease appears to be seed borne. The evidence at hand indicates that it does not live over winter in the field and is not transmitted by insects. It spreads rapidly in rainy weather.

A description is given of *Bacterium cucurbitae*, the causal organism.

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